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Chapter 1

MITOCHONDRIAL GENE DIVERSITY OF THE MEGA-HERBIVOROUS SPECIES OF THE GENUS *TAPIRUS* (TAPIRIDAE, PERISSODACTYLA) IN SOUTH AMERICA AND SOME INSIGHTS ON THEIR GENETIC CONSERVATION, SYSTEMATICS AND THE PLEISTOCENE INFLUENCE ON THEIR GENETIC CHARACTERISTICS

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ABSTRACT

We sequenced mitochondrial genes (*COI, COII, Cyt-b*) of accepted Latin America tapir species (*Tapirus pinchaque, T. terrestris* and *T. bairdii*) as well as an alleged new species, *T. kabomani*.

The mountain tapir (*T. pinchaque*) is a relatively rare large mammal species. Some population censuses indicate that no more than 2,000 mountain tapirs are left in the wilderness areas of Colombia, Ecuador and Peru. Our results showed that the gene

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diversity levels are medium to low with respect to other mammals sequenced for the same or similar genes. However, these gene diversity levels are not impoverished, which means that the genetic situation of this species is not as critical as its population censuses suggest. It will be crucial to determine the gene diversity levels in certain populations not included in the current work (the eastern and, possibly, western Andean Cordilleras in Colombia as well as the Tabaconas Namballe National Sanctuary in Peru), because they are probably the smallest populations of this species. On the other hand, the lowland tapir (*T. terrestris*), the species with the largest geographical distribution in Latin America, showed the highest gene diversity levels of all the other tapir species studied. Additionally, the genetic structure of *T. terrestris* is clearly more robust than that of *T. pinchaque*. Different geographic populations of both species showed different demographic trends throughout time. Our results including five samples of *T. kabomani* showed this taxon to be a haplogroup within *T. terrestris*, reducing the likelihood of *T. kabomani* being a new full species. Finally, we also analyzed the influence of diverse Pleistocene climatic changes on the mitochondrial haplotype diversification of *T. terrestris* and *T. pinchaque*. The Pleistocene Refugia and the Recent Lake hypotheses probably played integral roles in the evolutionary history of *T. terrestris.* In contrast, the Pleistocene Refugia hypothesis involving the Andes, which probably played an important part in the genetic diversification of other mammals, did not have a significant impact on *T. pinchaque*.

Keywords: Mitochondrial genes (*COI, COII* and *Cyt-b*), *Tapirus*, *T. pinchaque*, *T. terrestris*, *T. bairdii*, "*T. kabomani*," conservation genetics, speciation, Pleistocene biodiversity hypotheses

INTRODUCTION

Large mammals are often considered umbrella or emblematic species whose presence may greatly affect community structure (Meffe and Carrol, 1997). In general, the extraction of large vertebrates, especially those at the top of the trophic chain, can provoke strong extinction waves in complex natural systems (Pimm, 1991). These species can be considered as key to overall community structure because they live sympatrically with many other species. For example, East (1981) showed that the preservation of viable populations of the African wild dog (*Lycaon pictus*) and the cheetah (*Acinonyx jubatus*), due to their large hunting territories, helped to preserve the communities of other large African mammals.

In Latin America, three wild large perissodactyla species have been traditionally accepted: the Baird or Central America tapir (*Tapirus bairdii*), the mountain tapir (*T. pinchaque*) and the lowland or Brazilian tapir (*T. terrestris*).

The fossil evolution of the Tapiridae is old and very interesting. Perissodactyla is a very old group of mammals that arose around 60 Million years ago (MYA). In the fossil record, there are representative specimens from five main superfamilies (Tapiroidea, Rhinocerotoidea, Chalicotheroidea, Equoidea and Brontotheroidea) and 14 different families (Savage and Long, 1986; Holbrook, 1999). The order reached its maximum diversity peak during the Eocene and then declined from 14 to 4 families during the upper Oligocene (Radinsky, 1969; Froehlich, 1999; MacFadden, 1992; Metais et al., 2006). The Tapiridae family (Gray 1821) is currently composed of one unique genus, *Tapirus* (Brisson 1762). Colbert (2005) defined the Tapiridae family as the clade conformed by the most recent

common ancestor of *Protapirus.* The oldest record of *Tapirus* comes from the European Oligocene (33-37 MYA), where the fossil remains are found until the Pleistocene (McKenna and Bell, 1997). In North America, the *Tapirus* records indicate that they were present in the Middle Miocene through the present (Hulbert, 1995), whereas for Asia the records indicate that *Tapirus* has been in existence since the Lower Miocene (Deng, 2006). Currently, additional to the three quoted Latin American tapir species, there is a fourth species in Southern Eastern Asia, the Malayan tapir (*T. indicus*). Two of these species are exclusively distributed in South America and they are the subject of this work, from a genetics point of view. The mountain tapir (*T. pinchaque*) has a unique and critical role because it is an indispensable seed disperser in the Andean mountains (Colombia, Ecuador, Northern Peru) but its conservation status is in jeopardy because of its relatively low numbers (less than 2,500 individuals; Cavelier et al., 2010). Additionally, this is an umbrella species with an average territory size per tapir of 880 ha (Downer, 1996). This is a substantially large home range. Downer's work in the Sangay National Park suggested that a minimum of 1,000 mountain tapirs were needed to ensure a reproductively viable population. Such a population would need at least 293,500 ha. The necessary protection of this area would positively benefit many other sympatric animal species, like the Andean bear, puma, and various deer species (Downer, 1996). Indeed, the cloud forests of the Andean region, where the mountain tapir lives, contain some of the greatest organismic diversity on Earth (Brehm et al., 2005; Krömer et al., 2005), with a noteworthy number of endemic species (Kessler, 2002). However, about 90 % of the mountain Andean forests have been deforested (Henderson et al., 1991) and a great proportion of páramos has been transformed via burns into extensive ranchlands and croplands (potatoes) (Verweij, 1995). Additionally, around 83 % of Colombia's mountain forests have been highly affected by human activity (Cavelier and Etter, 1995). Besides ranching and agriculture, the mountain tapir is also highly threatened by poaching and illegal trade of its body parts (Downer, 2003). In Andean areas, the introduction of livestock has also spread new diseases and attracted more potential predators of the mountain tapir.

The lowland tapir has a wide geographical distribution across a great part of South America. From an ecological perspective, the lowland tapir is considered the "architect" of their Neotropical habitats because it is a very efficient disperser and seed predator (Wallace et al., 2010). Cabrera (1961) considered four morphological and geographical subspecies of *T. terrestris*: *T. t. colombianus, T. t. terrestris, T. t. aenigmaticus,* and *T. t. spegazzinni. T. t. colombianus* (Hershkovitz 1954; Type locality: El Salado, between Valencia and Pueblo Bello on the eastern slope of the Sierra Nevada de Santa Marta, in Magdalena, Colombia) inhabits Northern Colombia and around Maracaibo Lake in Venezuela. *T. t. terrestris* (Linnaeus 1758; Type locality: Pernanbuco, Brazil) lives in Venezuela, Guyana, Suriname, French Guyana, with its major fraction in Brazil (including Amazonas) up to the Misiones within Argentina. *T. t. aenigmaticus* (Gray 1872; Type locality: Macas, Eastern Ecuador) is distributed in Southeastern Colombia, Eastern Ecuador and Northern/Eastern Peru (Amazonian area). *T. t. spegazzinni* (Ameghino 1909; Type locality: Rio Pescado, Orán, Salta, Argentina) is distributed in Southeastern Brazil and Mato Grosso, Paraguay, Eastern Bolivia and Northwestern Argentina.

Recently, Cozzuol et al., (2013) claimed the existence of a new tapir species ("*T. kabomani*") in South America based on a comparison of mitochondrial Cytochrome-b (*Cyt-b*) gene sequences of four Amazon tapir individuals (two Brazilian animals sampled by these authors and two Colombian specimens sampled by the first author of the present study, M. R-

G) to the 45 *Cyt-b* gene sequences published by Thoisy et al., (2010). They also enclosed the results of two additional mitochondrial genes (mitochondrial Cytochrome Oxidase subunit I, *COI*, and subunit II, *COII*) for six *T. terrestris*, one *T. pinchaque* and three individuals of the alleged new species "*T. kabomani*." They concluded that the three mitochondrial genes supported "*T. kabomani*" as a full species. Voss et al., (2014), however, questioned the validity of "*T. kabomani*" as a different species from *T. terrestris*.

The current work provides some insight about the genetic structure and heterogeneity, demographic evolution, spatial structure, biological conservation, and systematics of the Latin American tapirs. Moreover, this work also explores the influence of the Pleistocene period on the Latin American tapirs. And, all of these insights are based on the analysis and study of mitochondrial genes *Cyt-b*, *COI*, and *COII*. These new data should be helpful in validating or rejecting the claim of a new species, "*T. kabomani*".

MATERIAL AND METHODS

Two sets of data were used in this study. The first set consisted of 93 Latin American tapir individuals analyzed for three specific concatenated mitochondrial genes (*Cyt-b + COI + COII*). They were the same genes analyzed by Cozzuol et al. (2013) and whose mitochondrial sequences are accessible in GenBank for the two Brazilian specimens they classified as an alleged new species "*T. kabomani*." Breaking the first group down by taxa, it had 46 *T. pinchaque*, 29 *T. terrestris*, 13 *T. bairdii*, and 5 *T. kabomani*. *T. pinchaque* was constituted by 26 from Ecuador representing three populations, Northern Ecuador, Sangay National Park and Podocarpus National Park, and the remainder of the 46 (20) was from Colombia representing three populations: Los Nevados National Park, Tolima and Purace National Park.

Another 29 of the first set were *T. terrestris*. Five of these individuals were from Ecuador, four were from Colombia, seven from Peru, three from Bolivia, three from Brazil, three from Surinam, and one was from Argentina. Three animals of the 29 were from American zoos in Cincinnatti, Milwakee and Columbus. Another 13 of the 93 were *T. bairdii*. Nine of these animals were from Panama, three were from Costa Rica and one was from Colombia. Five of the 93 were *T. kabomani*- including the two Brazilian specimens of Cozzuol et al., (2013).We detected three additional specimens with mitochondrial haplotypes of the alleged *T. kabomani*. There were from 1) San Martín de Amacayacu along the Amazon River in Colombia, 2) the Mazan River which is a tributary of the Napo River, in the Peruvian Amazon, and 3) near Tena along the upper Napo River in the Ecuadorian Amazon.

However, at least, two of these animals (the Colombian and the Ecuadorian ones) presented typical morphotypes of *T. terrestris*, although they showed "*T. kabomani*" haplotypes (see Figure 1). The Peruvian sample consisted of skin obtained from hunters and we could not determine the morphotype of the specimen.

a

b

Figure 1. An individual with morphotype of *Tapirus terrestris* but with "*T. kaboman*" mitochondrial haplotype from San Martin de Amacayacu, Amazon River, Colombian Amazon (A); An individual with morphotype of *Tapirus terrestris* but with "*T. kaboman*" mitochondrial haplotype from near to Tena, upper Napo River, Ecuadorian Amazon (B).

The second set of individuals contained 141 *T. terrestris*, sequenced at the *Cyt-b* gene. Forty-one of these came from different regions of Colombia. Of these, 1 was from Bajo Sinú-Tierra Alta, (Córdoba Department), 2 were from Mesay River (Caquetá Department), and another was from Fondo Canaima, (Vichada Department). Of the 41, 18 were also from Leticia to San Juan de Atacuarí (Amazonas Department), 7 from the Eastern Colombian Llanos (Meta Department), 3 from Pto. Inirida (Guania Department), 3 from Palomino River-Sierra Nevada de Santa Marta and 6 from the Antioquia Department. Five of the 141 were from Venezuela (El Zulia, Maracaibo). Eleven of the 141 were from French Guiana (Carnopi River). Seven animals were from Ecuador (4 from Limoncocha, Sucumbios and 3 from Coca, Sucumbios). Thirty animals were from Peru [(one from Arica (Curaray River), 2 from Napo River (Nueva Vida and Mazán), 7 from Nanay River, one from Requena (Ucayali River), one

from Bretaña (Canal del Puhinauva-Ucayali River), 4 from Pucallpa (Ucayali River), and 15 from Pto. Maldonado (Madre de Dios River)]. Eleven animals were from Bolivia (9 from Mamoré River, one from Chimoré River and one from Villa Bella at the Beni River). Twenty four animals were from Brazil [2 from Yavarí River, 12 from Tabatinga, 2 from Negro River, 2 from Santarem (Pará state) and 6 from the Amazon's mouth (Pará state)]. One animal was from Paraguay (from Hernandarias). And, 4 animals were from Argentina (the Yungas area in Salta-Jujuy). Additionally, one animal was from the Barcelona Zoo (Spain) and 5 animals were from US zoos. One animal of unknown origin was also analyzed.

Molecular Procedures

DNA from teeth, bones, muscle, and skins, were obtained with the phenol-chloroform procedure (Sambrook et al., 1989), whereas DNA samples from hair and blood were obtained with 10% Chelex® 100 resin (Walsh et al., 1991). Amplifications for *Cyt-b* gene were achieved using primers L7 (5' ACC AAT GAC ATG AAA AAT CAT CGT T 3') and H6 (5' TCT CCA TTT CTG GTT TAC AAG AC 3'), that had been designed for perissodactyles (Tougard et al., 2001). The PCR reactions were performed in a 50-µl volume, including 10 µl of 10x Buffer, 7 µl of 3 mM $MgCl_2$, 2 µl of 10 mM dNTPs (dNTP Mix Promega), 4 µl (15 pmol) of each primer, one unit of Taq DNA polymerase (genTaq polimerasa), 2 µl of DNA from blood, skin or muscle tissue (50-200 ng/µl) or 4-10 µl of DNA from hair and teeth (6-35 ng/µl) and a variable quantity of ddH_20. PCR reactions were carried out in a Geneamp PCR system 9600 (Perkin Elmer) and in an iCyclerTM BioRad thermocycler. We used the following temperatures: 94 °C for 5 minutes, 35 cycles of 50 s at 94 °C, 50 s at 53 °C and 1.5 minutes at 72 °C and a final extension time for 10 minutes at 72 °C. For the *COI* and *COII* genes, the amplification conditions followed Ashley et al., (1996) and Hebert (2003, 2004). All amplifications, including positive and negative controls, were checked in 2 % agarose gels, employing the molecular weight marker ϕX174 DNA digested with *Hind* III, *Hinf* I and HyperLadder IV. The gels were visualized in a Hoefer UV Transilluminator. Those samples that amplified were purified using membrane-binding spin columns (Qiagen). The double-stranded DNA was directly sequenced in a 377A (ABI) automated DNA sequencer. The samples were sequenced in both directions and all the samples were repeated to ensure sequence accuracy.

Data Analyses

Genetic Diversity, Linkage and Heterogeneity Analyses

The sequences were edited and aligned with BioEdit Sequence Alignment Editor (Hall, 2004) and DNA Alignment (Fluxus Technology Ltd).

We used the following statistics to determine the genetic diversity at the three concatenated mitochondrial genes for *T. terrestris*, *T. pinchaque*, *T. bairdii* and the alleged "*T. kabomani*": the number of polymorphic sites (S), the number of haplotypes (H), the haplotypic diversity (H_d), the nucleotide diversity (π), the average number of nucleotide differences (k) and the θ statistic by sequence.

The possible linkage disequilibrium within the *Cyt-b* gene for *T. terrestris* was evaluated by two different statistics. We obtained the average value of R^2 for all the comparison pairs among nucleotide sites (ZnS) (Kelly, 1997) as well as among the adjacent polymorphic nucleotide sites (Za), and ZZ (Rozas et al., 2001). This is equal to Za - ZnS, using the software DNAsp 5.10 (Librado and Rozas, 2009).

Different tests were carried out to measure genetic heterogeneity, and possible gene flow estimates, among the four Latin American tapir taxa studied. These tests were those of Hudson et al., (1992a,b) (H_{ST} , K_{ST} , K_{ST}*, Z, Z*), Hudson (2000)'s Snn test and the chi-square test on the haplotypic frequencies with permutation tests using 10,000 replicates. We also estimated the G_{ST} statistic from the haplotypic frequencies and the γ_{ST}, N_{ST} and F_{ST} statistics (Hudson et al., 1992a) from the nucleotide sequences.

We carried out two different AMOVA analyses at the *Cyt-b* gene for *T. terrestris* to determine if the gene diversity within this species was distributed in different hierarchical geographical levels (Excoffier et al., 1992). The first analysis was carried out taking into account the six haplogroups found in the phylogenetic analysis clustered in three main population sets: northern, Amazonian, and southern. Thus, this analysis was applied to the overall geographical *T. terrestris* range analyzed. The second analysis was completed taking into account six different geographical areas within the Amazon basin: 1-Northwestern Amazon in Colombia and Brazil, 2- Central and Eastern Brazilian Amazon, 3-Napo River and a tributary in Peru and Ecuador, 4- Ucayali River in Peru, 5-Madre de Dios River in Peru and 6- Mamore River and a tributary in Bolivia. These six geographical areas were clustered into two main groups: northern Amazon (the first three areas) and southern Amazon (the last three areas). This analysis was carried out to determine the gene diversity structure in the Amazon region. The fixation indices of Wright (1951) were estimated: Φ_{sc} (variation of populations within the groups), Φ_{ct} (variation among groups) and Φ_{st} (variation among individuals). These analyses were carried out by means of the software ARLEQUIN 3.0 (Excoffier et al., 2005).

Phylogenetic Analyses

The Modeltest (Posada and Crandall, 1998) and the Mega 5.1 software (Tamura et al., 2011) were applied to determine the best evolutionary nucleotide model for the analyzed concatenated gene sequences for all the *Tapirus* taxa studied.

To determine if *T. pinchaque* or *T. terrestris*, contained a more conspicuous genetic structure, we used a consensus maximum parsimony (MP) tree for the three concatenated mitochondrial genes. We also obtained a maximum likelihood (ML) tree to determine how many significant clades were within *T. terrestris*, at the *Cyt-b* gene. In this analysis, 80 *T. terrestris* haplotypes were considered (including two haplotypes later classified as "*T. kabomani*"). To carry out this task, we tested the hypothesis that the *T. terrestris* sequences fall into one to 10 different groups. These classifications were contrasted with the ML tree. For this, we performed parametric bootstrapping and a posteriori significance test with the Swofford-Olsen-Waddell-Hillis test (SOWH; Huelsenbeck and Bull, 1996; Swofford et al., 1996). The 10 hypotheses were used as a model tree for parameter estimation and for generating 100 replicate data sets in the software Seq-Gen 1.2.5 (Rambaut and Grassly, 1997) which presented a uniform base composition. Goldman et al., (2000) demonstrated that this procedure can increase power in rejecting the null hypothesis and is better than typical

nonparametric tests for comparisons of a posteriori hypotheses. The same was performed with the Shimodaira and Hasegawa (1999) test (a nonparametric SH test).

We conducted two additional phylogenetic trees. One was a ML tree with the 93 tapirs sequenced at the three concatenated mitochondrial genes to determine the relationship of the five "*T. kabomani*" with regard to the other tapir taxa. The second was an ML tree with some animals only sequenced at the *COI* gene since this gene is used as a barcode to discriminate different species (Herbert et at., 2003, 2004). Similarly, we estimated the Kimura 2P genetic distance (Kimura, 1980), for the three mitochondrial genes, among all the Neotropical *Tapirus* taxa because this genetic distance is a standard measurement for barcoding tasks (Hebert et al., 2003, 2004).

Possible divergence times were obtained using a Median Joining Network (MJN) (Bandelt et al., 1999). These were applied to all the haplotypes of the three concatenated mitochondrial genes for all the tapir taxa considered as well as to the *T. terrestris* haplotypes at the *Cyt-b* gene. These were constructed with Network 4.6 software (Fluxus Technology Ltd). The ρ statistic (Morral et al., 1994) was estimated and transformed into years. The standard deviation of ρ was also calculated (Saillard et al., 2000), which is unbiased and highly independent of past demographic events. Thoisy et al., (2010) used two different mutation rates: 5.6×10^{-3} and 2.5×10^{-2} substitutions/site/million years respectively. Relative to our work with the genus *Tapirus*, the first rate translated into around one mutation each 537,634 years. The second rate was equal to about one mutation each 120,482 years. This last mutation rate agrees quite well with that reported by Nabholz et al., (2008, 2009) for mammals at the *Cyt-b* gene. We employed an intermediate value of 1.5×10^{-2} substitutions/site/million years (around one mutation each 200,000 years), closer to the second value used by Thoisy et al., (2010).

Demographic Changes

We used two methods to determine possible demographic changes across the natural history of *T. pinchaque*, *T. terrestris* and "*T. kabomani*" for the three concatenated mitochondrial genes as well as for the *Cyt-b* gene in the lowland tapir. (1) Following the method of Rogers and Harpending (1992) and Rogers et al., (1996) we used a mismatch distribution (pairwise sequence differences). The raggedness *rg* statistic (Harpending et al., 1993; Harpending, 1994) was used to determine the similarity between the observed and the theoretical curves. (2) We used the Fu and Li D and F tests (Fu and Li, 1993), the Fu F_S statistic (Fu, 1997), the Tajima D test (Tajima, 1989) and the R_2 statistic (Ramos-Onsins and Rozas, 2002) to determine possible changes in population size (Simonsen et al., 1995; Ramos-Onsins and Rozas, 2002). All of these statistics and tests were obtained by means of the DNAsp 5.10 and Arlequin 3.0 programs.

Spatial Structure

Garnering this information can assists in our understanding of the evolutionary events that have determined the natural history of the tapir species. A Mantel's test (Mantel, 1967) was used to detect possible overall relationships between a genetic matrix among individuals (Log-Det genetic distance; Nei and Kumar, 2000) and the geographic distance matrix among the individuals analyzed. In this study, Mantel's statistic was normalized according to Smouse et al., (1986). This procedure transforms the statistic into a correlation coefficient. The geographic distances were measured with the Spuhler's (1972) procedure, where D =

arcos (cos $X_{(i)}$. cos $X_{(j)}$ + sin $X_{(i)}$. sin $X_{(j)}$ cos $|Y_{(i)} - Y_{(j)}|$), where $X_{(n)}$ and $Y_{(n)}$ are the latitude and longitude of the *nth* individual sampled, respectively. The significance of the correlations obtained was tested using a Monte Carlo simulation with 1,000 permutations. This test was undertaken by means of the software NTSYS v. 2.1 (Rohlf, 2000). This analysis was carried out for *T. pinchaque*, *T. bairdii* and the overall sample of *T. terrestris* for the three concatenated mitochondrial genes as well as for the different haplogroups found and for different geographical regions at the *Cyt-b* gene for *T. terrestris*. For all the Amazon *T. terrestris* individuals sequenced at the *Cyt-b* gene, an AIDA analysis was used (Bertorelle and Barbujani, 1995). The expressions of the respective AIDAs coefficients are as follows:

$$II = (n \Sigma_{i=1 \text{ to } n} \Sigma_{j > i \text{ to } n} w_{ij} \Sigma_{k=1 \text{ to } S} (p_{ik} - p_k)(p_{jk} - p_k)) / (W \Sigma_{i=1 \text{ to } n} \Sigma_{k=1 \text{ to } S} (p_{ik} - p_k)^2)$$

and

$$cc = ((n - 1) \Sigma_{i=1 \text{ to } n} \Sigma_{j > i \text{ to } n} w_{ij} \Sigma_{k=1 \text{ to } S} (p_{ik} - p_{jk})^2) / (2W \Sigma_{i=1 \text{ to } n} \Sigma_{k=1 \text{ to } S} (p_{ik} - p_k)^2)$$

where n is the sample size, W is the number of pairwise comparisons of a distance class given, p_{ik} and p_{jk} are the haplotypes of the *ith* and *jth* individuals, respectively. At the *kth* nucleotide site, p_k is the *kth* element of the average vector and w_{ij} is one if individuals *i* and *j* are within the same distance class; otherwise it is zero. Summation includes the Σ nucleotide sites for all the *n* individuals analyzed. To connect the individuals sequenced within each distance class, the Gabriel-Sokal network (Gabriel and Sokal, 1969; Matula and Sokal, 1980) and the Delaunay's triangulation with elimination of the crossing edges (Ripley, 1981; Upton and Fingleton, 1985; Isaaks and Srivastava, 1989) were used. However, the results were very similar in each case. The Bonferroni (Oden, 1984), Oden's Q and the Kooijman's tests were estimated to determine the statistical significance of the autocorrelation coefficients.

RESULTS

Gene Diversity, Genetic Heterogeneity and Phylogenetic Considerations for the Latin American Tapir Taxa

The GTR with gamma distributed rate variation among sites for both the maximum likelihood and the AIC criteria was the best fit nucleotide substitution model at the three concatenated genes.

Out of 4,753 comparisons among the total polymorphic sites at the *Cyt-b* gene in *T. terrestris*, only 303 comparisons were statistically significant by means of a Fisher exact test (6.4 %), but they were not different from the Type I error of 5 %. Similarly, the values of the statistics of Kelly (1997) (ZnS = 0.0219; 95 % of Confidence interval, CI = 0.01498 – 0.31650), of Rozas et al., (2001) (Za = 0.0244; CI = 0.01706 – 0.32607) and ZZ = 0.0026 (CI = -0.05363 – 0.05625) also showed that there was no significant association among the polymorphic sites at this mitochondrial gene. Therefore, no evidence of linkage among

polymorphic sites was detected at this mitochondrial gene. Out the four taxa of *Tapirus* considered at the three concatenated mitochondrial genes, clearly *T. terrestris* showed the highest levels of gene diversity (H_d = 0.985; π = 0.0094; k = 22.98), followed by *T. pinchaque* (H_d = 0.957; π = 0.0041; k = 9.98). In contrast, "*T. kabomani*" (H_d = 0.900; π = 0.0033; k = 8.00) and *T. bairdii* (H_d = 0.910; π = 0.0017; k = 4.08) yielded the lowest levels of gene diversity (Table 1). The "*T. kabomani*" gene diversity should be enclosed within the gene diversity of *T. terrestris* (proof 1). The genetic heterogeneity among these four taxa was highly significant and of a large magnitude for all the statistics used (for instance, γ_{ST} = 0.808 and F_{ST} = 0.903; Table 2a), with the associated indirect gene flow values practically non-existent (Nm = 0.05-0.12).

Table 1. Gene diversity statistics for *Tapirus terrestris, T. pinchaque, T. bairdii* and the alleged new species "*T. kabomani*" at the mitochondrial genes sequenced (*Cyt-b* + *COI* + *COII*). The statistics estimated were the number of haplotypes (NH), the haplotypic diversity (H_d), the nucleotide diversity (π), the average number of nucleotide differences (K) and the θ statistic (= $2N_e\mu$; N_e = effective female population size; μ = mutation rate per generation) by sequence

	NH	H_d	π	K	θ per sequence
Tapirus terrestris	24	0.985 ±0.014	0.0094 ±0.0007	22.985 ±10.42	29.538 ±9.448
Tapirus pinchaque	31	0.957 ±0.021	0.0041 ±0.0005	9.981 ±4.648	13.652 ±4.161
"Tapirus kabomani"	4	0.900 ±0.161	0.0033 ±0.0009	8.000 ±4.483	7.680 ±4.165
Tapirus bairdii	9	0.910 ±0.068	0.0017 ±0.0003	4.077 ±2.173	5.156 ±2.268

Table 2. Genetic heterogeneity statistics applied to Latin American tapirs: among four Latin American tapir taxa (*T. pinchaque, T. terrestris, T. bairdii* and the alleged new species "*T. kabomani*") (A); between *T. pinchaque* and *T. terrestris* (B). *Significant Probability (P < 0.01)

A
Among four Latin American tapir taxa

Genetic differentiation estimated	P	Gene flow	
χ^2 = 279.000 df = 201	0.0002*		
H_{ST} = 0.0287	0.0000*	γ_{ST} = 0.8079	Nm = 0.12
K_{ST} = 0.8026	0.0000*	N_{ST} = 0.9078	Nm = 0.05
K_{ST}* = 0.3433	0.0000*	F_{ST} = 0.9034	Nm = 0.05
Z_S = 941.8106	0.0000*		
Z_S* = 6.4554	0.0000*		
S_{nn} = 1.0000	0.0000*		

B

***T. pinchaque* sample vs. *T. terrestris* sample**

Genetic differentiation estimated	P	Gene flow	
$\chi^2 = 104.041$ df = 70	0.0052*	$G_{ST} = 0.0411$	Nm = 11.68
$H_{ST} = 0.0414$	0.0004*	$\gamma_{ST} = 0.4389$	Nm = 0.64
$K_{ST} = 0.4167$	0.0000*	$N_{ST} = 0.5158$	Nm = 0.47
$K_{ST}^* = 0.2421$	0.0000*	$F_{ST} = 0.5145$	Nm = 0.47
$Z_S = 678.8785$	0.0000*		
$Z_S^* = 6.1920$	0.0000*		
$S_{nn} = 0.7876$	0.0000*		

In another heterogeneity analysis, *T. pinchaque* was only analyzed with regard to *T. terrestris* (Table 2b). In this case, the seven genetic heterogeneity tests were also highly significant at the $P < 0.05$ level. The relative genetic differentiation tests yielded elevated amounts of genetic heterogeneity between the *Tapirus* taxa ($\gamma_{ST} = 0.439$ and $F_{ST} = 0.514$). The gene flow estimates were clearly lower than 1 (Nm = 0.47-0.64), which showed that these taxa are genetically disconnected. Although, "*T. kabomani*" showed significant differences with *T. terrestris* as well as with *T. pinchaque*, the genetic differences with *T. terrestris* are considerably lower than with *T. pinchaque* (six significant heterogeneity tests, $\gamma_{ST} = 0.151$ and $F_{ST} = 0.454$, Nm = 2.82-0.60 versus seven significant heterogeneity tests, $\gamma_{ST} = 0.300$ and $F_{ST} = 0.711$, Nm = 1.16-0.20, respectively). Therefore, "*T. kabomani*" is less differentiated genetically speaking from *T. terrestris* than from *T. pinchaque* and it showed less differentiation in regard to *T. terrestris* than the genetic differentiation between *T. terrestris* and *T. pinchaque*. This provides strong evidence (proof 2) that "*T. kabomani*" is more closely related to *T. terrestris* than to the other tapir species, which disagrees with the point of view of Cozzuol et al., (2013).

The genetic structure within *T. terrestris* is much more robust (greater) than within *T. pinchaque*. This is verified by the consensus MP tree (Figure 2). *T. pinchaque* showed some small geographical clusters with several individuals of nearby geographical regions. Within *T. pinchaque* there were five Colombian individuals (four from Los Nevados National Park and one from Gaitania, Tolima) and two individuals from the Alto Papallacta River in the Napo Province in Ecuador. It also contained seven Ecuadorian individuals (six from the Napo Province and one from Sangay National Park), two from the Napo Province and an Ecuadorian cluster of six.

The Ecuadorian cluster represented three different Ecuadorian populations. Two *T. pinchaque* sequences were more related to those from *T. terrestris* than to those of the other mountain tapirs. These were the cases of one Colombian individual from La Planada, Tolima (Colombia) and one Ecuadorian individual from Chaco, Oyacachi, Napo. However, the relationships among the *T. pinchaque* were considerably less structured than those observed within *T. terrestris*. This second species was composed by more robust haplogroups than those detected in the mountain tapir. Even an exact probability test with a Markov chain length of 100,000 steps only revealed one significant population comparison pair: Los Nevados vs. Sangay National Park ($p = 0.0297 \pm 0.0013$) out of the six populations of *T. pinchaque* studied. This may indicate that (proof 3) *T. terrestris* is an older species than *T. pinchaque*.

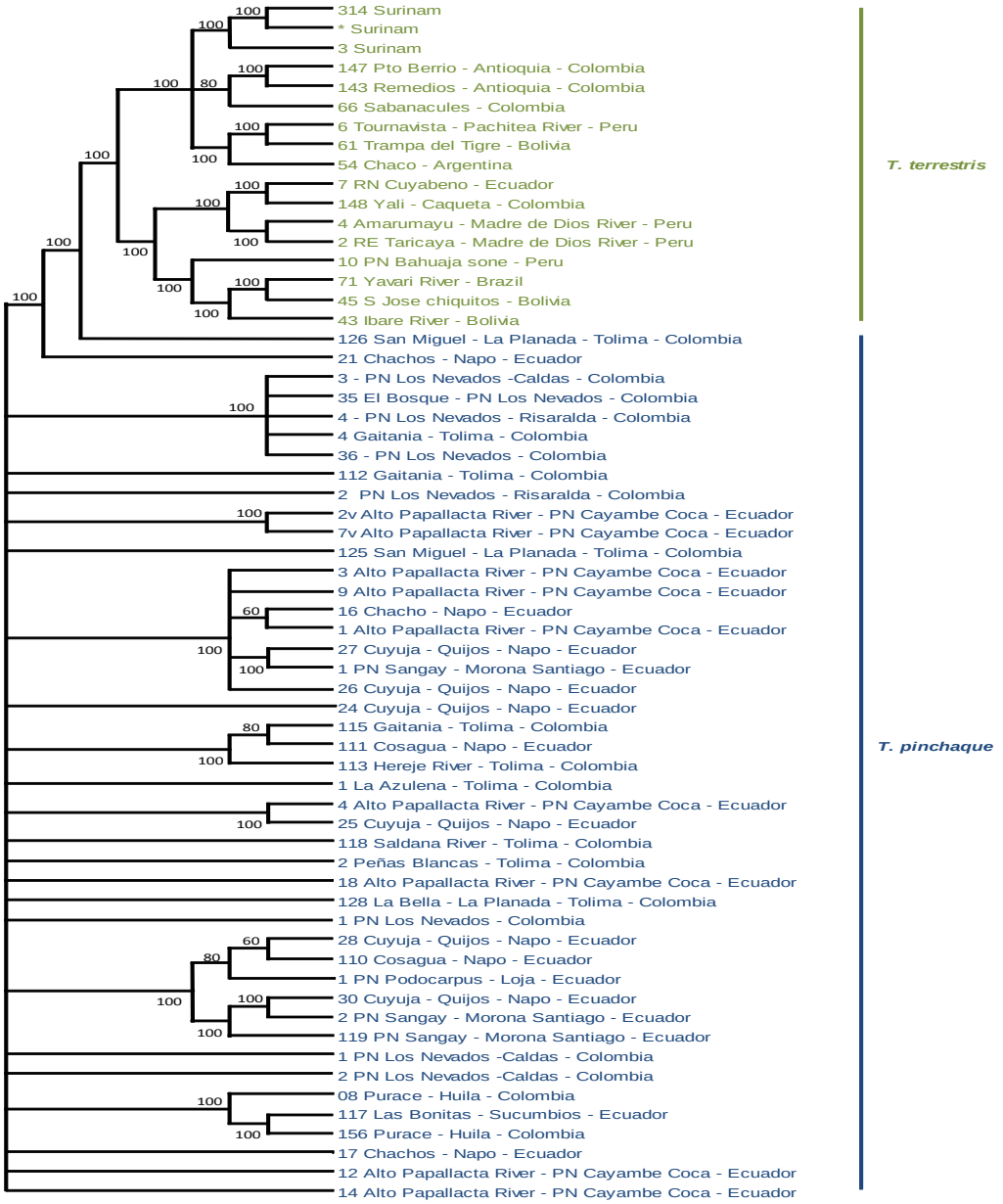

Figure 2. Consensus maximum parsimony tree with 45 *Tapirus pinchaque*, plus 17 *T. terrestris* by using the concatenated sequences of three mitochondrial genes (*Cyt-b + COI + COII*).

The most developed genetic structure in *T. terrestris* can be seen in the AMOVA analyses carried out. The first AMOVA, with the six haplogroups, showed that the major part of the genetic variance was among lineages (64%; Φ_{sc} = 0.762, P-value = 0.000±0.00), followed by the gene variance among the individuals (36%; Φ_{st} = 0.641, P-value = 0.000 ± 0.000). In contrast, the genetic variance among the three main groups (North, Amazonian and South) did not show significant variance (0%, Φ_{ct} = -0.510, P-value = 1.000 ± 0.000). However, in the second AMOVA, by geographical areas within the Amazon basin, the major

part of the genetic variance was explained by the difference among the individuals (90%, Φ_{st} = 0.499, P-value = 0.000 $\pm$ 0.000). The genetic variance among the populations, although significant, only explained 10 % of the genetic variance (Φ_{sc} = 0.148, P-value = 0.000 $\pm$ 0.000). The gene variance between the two main groups was again not significant (0%, Φ_{ct} = -0.057, P-value = 0.913 $\pm$ 0.008).

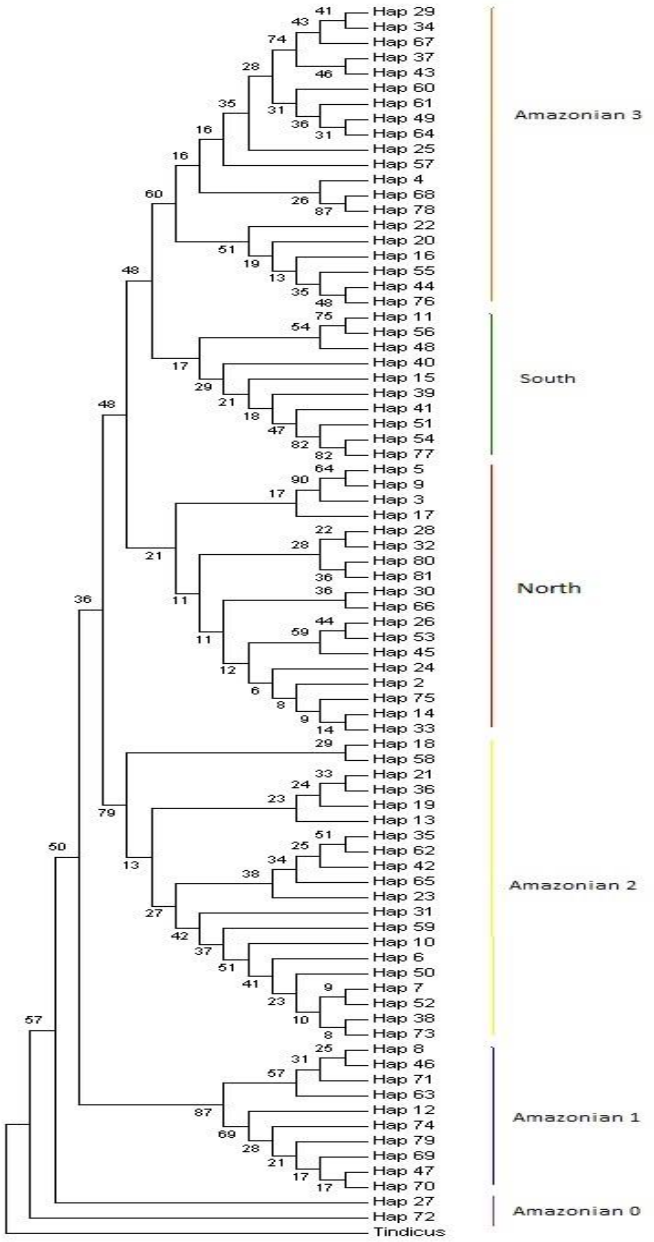

Figure 3. Maximum likelihood (ML) tree with the 80 haplotypes (from haplotype 2 to haplotype 81) found at the *Cyt-b* gene (1,140 bp) for the 141 lowland tapirs sequenced analyzed. Numbers in the nodes are bootstrap percentages.

The lower gene variance among geographical regions relative to among haplogroups is explained because the diverse haplogroups coexisted sympatrically at the same points of the Northern Peruvian Amazon (four out of six different haplogroups). This was also true for the Colombian Amazon (six of six), Northwestern Brazilian Amazon (three out of six), Southern Peruvian Amazon (three out of six) and Bolivian Amazon (three out of six). The existence of extremely, well-differentiated haplogroups within *T. terrestris* could help us to understand why "*T. kabomani*" is an additional haplogroup within "*T. kabomani*" rather than a full species (proof 4). An ML tree, applied to *T. terrestris* at the *Cyt-b*, showed that six haplogroups were detected within this species (Figure 3). The SOWH tests (and also the SH tests) indicated that there was neither support for the taxonomic schemes of 1 to 5 clusters nor for the 7 to 10 clusters. The maximum likelihood trees were significantly different at the 0.001 level (since 12,569 to 689,999 log likelihood units). However, the scheme suggested that six clusters did not significantly deviate from the tree we obtained (1,455 log likelihood units, $p < 0.55$).

Table 3 shows the Kimura 2P genetic distances among all of the Neotropical *Tapirus* taxa. For all three genes, "*T. kabomani*" yielded lower genetic distances with regard to *T. terrestris* than did *T. pinchaque* (*Cyt-b*: 0.018 ± 0.003 vs. 0.024 ± 0.003; *COI*: 0.005 ± 0.002 vs. 0.010 ± 0.004; *COII*: 0.013 ± 0.003 vs. 0.017 ± 0.004, respectively). The genetic distances between *"T. kabomani"* and *T. terrestris*, typical of different populations within a species, are not surprising results. However, the small genetic distances between what are traditionally considered full species (*T.terrestris* and *T. pinchaque*) are (proof 5).

Table 3. Kimura 2P genetic distance (Kimura, 1980), in percentages, with standard deviations among Neotropical *Tapirus* taxa pairs for three mitochondrial genes (*COI, COII* and *Cyt-b*)

COI				
Taxa	T. terrestris	"T. kabomani"	T. pinchaque	T. bairdii
T. terrestris	-			
"T. kabomani"	0.5 % ± 0.2	-		
T. pinchaque	1.0 % ± 0.4	1.2 % ± 0.4	-	
T. bairdii	5.8 % ± 1.0	6.0 % ± 1.0	5.9 % ± 1.0	-
COII				
Taxa	T. terrestris	"T. kabomani"	T. pinchaque	T. bairdii
T. terrestris	-			
"T. kabomani"	1.3 % ± 0.3	-		
T. pinchaque	1.7 % ± 0.4	1.2 % ± 0.4	-	
T. bairdii	7.8 % ± 1.1	7.7 % ± 1.0	8.0 % ± 1.1	-
Cyt-b				
Taxa	T. terrestris	"T. kabomani"	T. pinchaque	T. bairdii
T. terrestris	-			
"T. kabomani"	1.8 % ± 0.3	-		
T. pinchaque	2.4 % ± 0.3	2.3 % ± 0.4	-	
T. bairdii	12.5 % ± 1.2	12.1 % ± 1.2	12.4 % ± 1.1	-

Figure 4 shows the ML tree for the three concatenated mitochondrial genes with the "*T. kabomani*" sequences (both Brazilian individuals reported by Cozzuol et al., 2013 and three specimens sampled by us). Clearly, our tree with 93 specimens showed reciprocal monophyly between *T. terrestris* and *T. pinchaque.* Moreover, the alleged "*T. kabomani*" is,

mitochondrially speaking, a clade within *T. terrestris*. Thus, our results do not provide positive data in favor of *T. kabomani* as a new and full tapir species (proof 6). The ML tree with only the barcoding gene *COI* employed for species discrimination, also showed "*T. kabomani*" as a clade within *T. terrestris* (Figure 5; proof 7). This demonstrates reciprocal monophyly between *T. terrestris* and *T. pinchaque*.

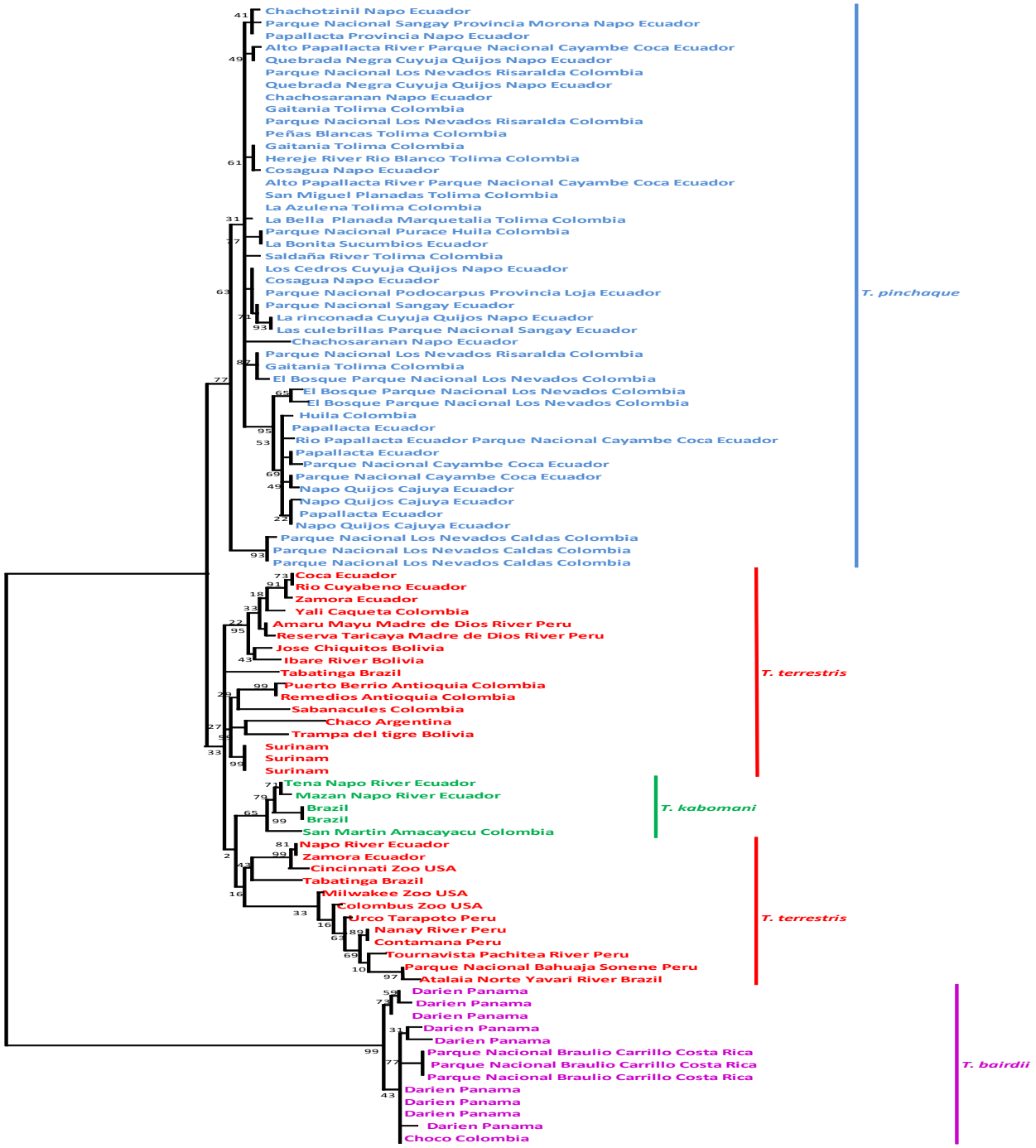

Figure 4. Maximum likelihood (ML) tree for 93 Neotropical tapir specimens (including *T. pinchaque, T. terrestris, T. bairdii* and the alleged new species, "*T. kabomani*", reported by Cozzuol et al., 2013) for three concatenated mitochondrial genes (*Cyt-b + COI + COII*). *T. kabomani* was within the *T. terrestris* clade and therefore these mitochondrial genes did not provide positive evidence for *T. kabomani* as a full species.

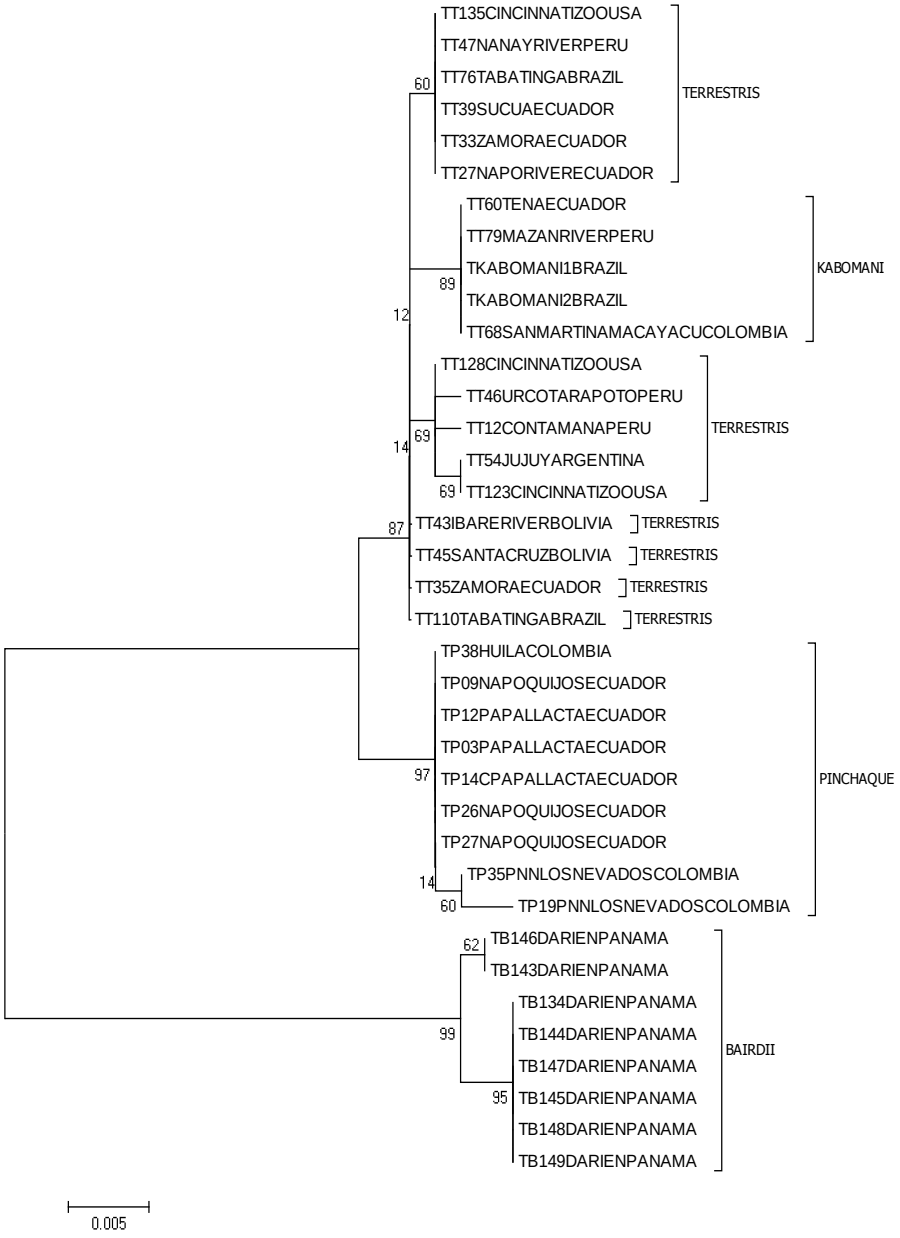

Figure 5. Maximum likelihood (ML) tree for different individuals of the three Neotropical tapir species recognized and the alleged new species reported as "*T. kabomani*" by Cozzuol et al., (2013) at the *COI* gene. "*T. kabomani*" was a clade within *T. terrestris*.

The MNJ procedure including the four possible Latin American tapir taxa for the three concatenated genes (Figure 6) showed that the haplotype sets of *T. bairdii*, *T. terrestris* and *T. pinchaque* are well defined. However, the "*T. kabomani*" haplotypes seem to be an extreme extension from those of *T. terrestris* (proof 8).

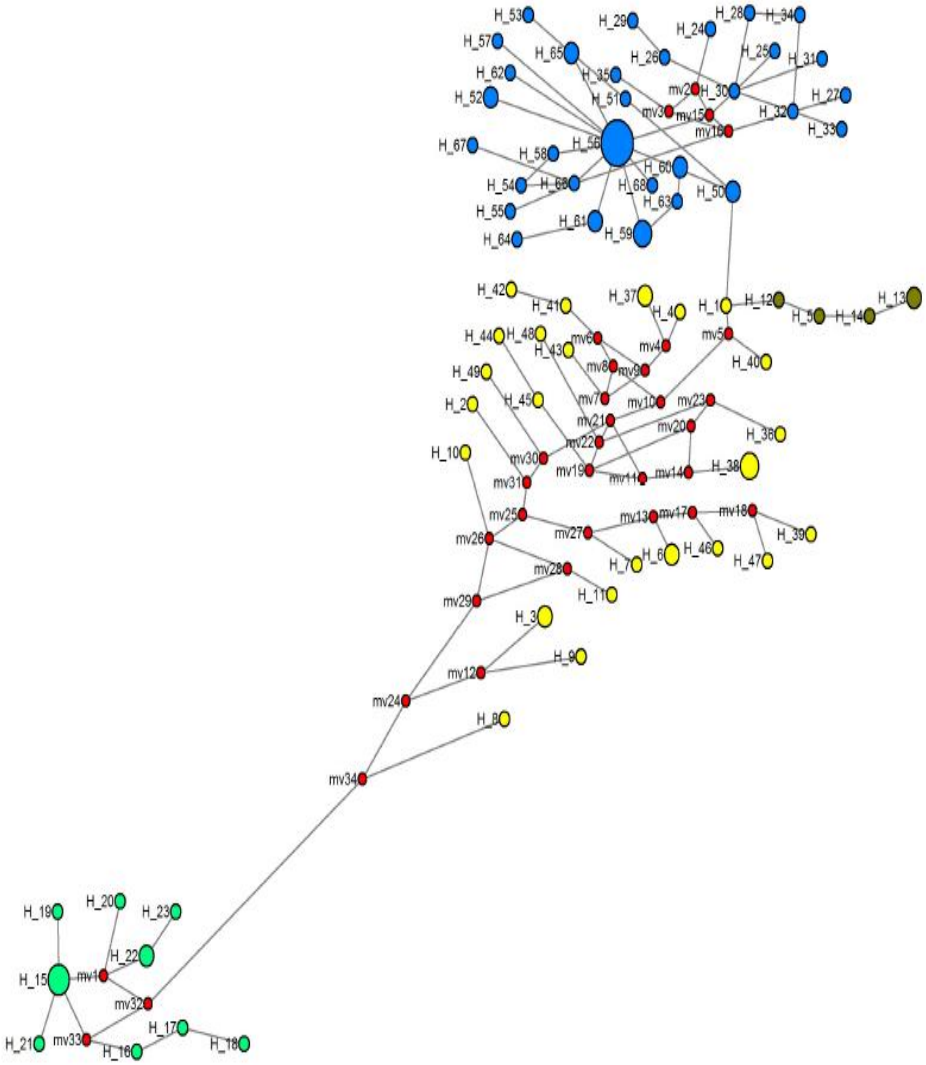

Figure 6. Median Joining Network (MJN) with the mitochondrial haplotypes found for *Tapirus bairdii* (green), *T. terrestris* (yellow), "*T. kabomani*" (brown) and *T. pinchaque* (blue) with the concatenated sequences of three genes (*Cyt-b* + *COI* + *COII*). Red circles are intermediate haplotypes not found. "*T. kabomani*" was a prolongation of *T. terrestris*.

An individual of *T. terrestris* from the Chiquitania in Bolivia (this was not reflected in any phylogenetic tree) connected the "*T. kabomani*" and *T. pinchaque* haplotypes. Some temporal split estimations were estimated by this procedure and they are interesting and cited here. For example, the temporal split between *T. bairdii* and *T. terrestris* and *T. bairdii* and

"*T. kabomani*" were 7,960,000 ± 69,282 YA (ρ = 39.8 ± 0.346) and 9,560,000 ± 144,222 YA (ρ = 47.8 ± 0.721), respectively. Therefore, on average, the time split between *T. bairdii* and *T. terrestris* + *"T. kabomani"* was around 8.76 MYA. The temporal split between *T. terrestris* and *T. pinchaque* was around 3,420,000 ± 509,117 YA (ρ = 17.1 ± 2.545) and the haplotype diversifications within both species were 3,300,000 ± 141,421 YA (ρ = 16.5 ± 0.707) and 1,208,696 ± 194,635 YA (ρ = 6.04 ± 0.973), respectively. In consideration of these data, the mitochondrial diversification of *T. terrestris* preceded *T. pinchaque* and/or being the subsistence of the original haplotypes in *T. terrestris* higher than in *T. pinchaque*. This last possibility agrees quite well with the fact that the gene diversity is lower in *T. pinchaque* than in *T. terrestris*. This is probably more due to the greater action in gene drift associated with considerably small population sizes in the first species rather than in the second species. The temporal split between *T. terrestris* and *"T. kabomani*" was around 1,300,000 ± 360,555 YA (ρ = 6.5 ± 1.802), considerably lower than that between *T. terrestris* and *T. pinchaque*, ratifying "*T. kabomani*" as a clade within *T. terrestris* (proof 9).

The MJN applied only to *T. terrestris* at the *Cyt-b* gene showed the following picture (Figure 7). There were six well-defined haplogroups. Amazon 0 (which corresponds to "*T. kabomani*" in the other analyses) and Amazon I haplogroups were the most differentiated ones. The average temporal split among the six haplogroups was around 1.6 ± 0.34 MYA. The beginnings of the temporal diversification within each haplogroup were as follows: 0.59 MYA for the Amazon I, 0.42 MYA for Amazon II and Amazon III, 0.55 MYA for the North and 0.33 MYA for the South.

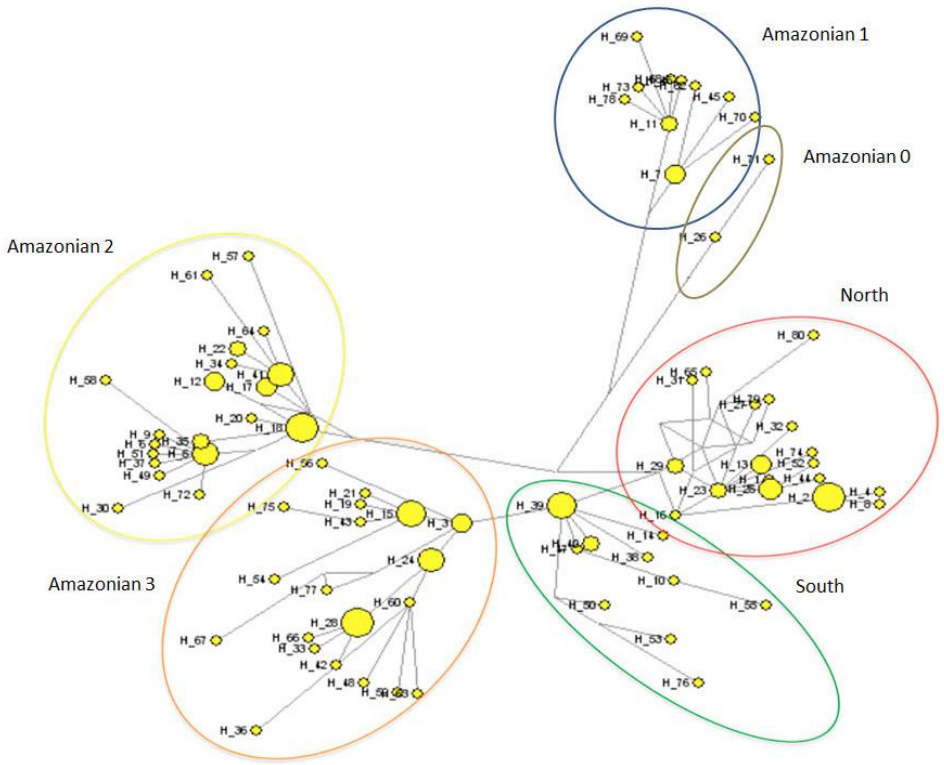

Figure 7. Median Joining Network (MJN) with the 80 haplotypes found at the *Cyt-b* gene for the 141 lowland tapirs analyzed. Six different mitochondrial haplogroups were found (Amazon 0, I, II, III, North and South). The Amazon 0 haplogroup corresponds to "*T. kabomani*". Red circles indicate missing intermediate haplotypes.

Demographic Changes in Latin American Tapirs

The mismatch distribution for *T. pinchaque* (Figure 8) showed no clear demographic changes of the total sample or in the Colombian population. But, it did show a significant curve related to a population expansion for the Ecuadorian sample (*rg* = 0.006, p = 0.011). For the Ecuadorian population, this expansion began around 2,900 years ago and the initial population size could have been around 4,770-6,691 females. Four of the five demographic change tests (Tajima'D, Fu & Li D* and F*, Fu's F) were significant for the total sample. However, the Ramos-Onsins & Rozas R2 test was not significant for any of the three samples (Table 4).

Table 4. Demographic statistics applied to the overall *Tapirus pinchaque* sample studied, to the Colombian sample and to the Ecuadorian sample. $^{+}$ P < 0.05; * P < 0.01, significant population expansions

	Tajima D	Fu & Li D*	Fu & Li F*	Fu's Fs	raggedness rg	R2
Overall *Tapirus pinchaque* sample	P[D ≤ -2.502] = 0.0000*	P[D* ≤ -4.49] = 0.0008*	P[F* ≤ -4.49] = 0.0006*	P[Fs ≤ -7.02] = 0.0032*	P[rg ≤ 0.0251] = 0.1213	P[R2 ≤ 0.0814] = 0.2091
Colombian *T. pinchaque* sample	P[D ≤ -2.279] = 0.0013*	P[D* ≤ -2.337] = 0.0263^{+}	P[F* ≤ -2.551] = 0.0240^{+}	P[Fs ≤ -6.126] = 0.0074*	P[rg ≤ 0.0363] = 0.2276	P[R2 ≤ 0.0923] = 0.0917
Ecuadorian *T. pinchaque* sample	P[D ≤ -1.825] = 0.0144^{+}	P[D* ≤ -2.337] = 0.0253^{+}	P[F* ≤ -2.551] = 0.0227^{+}	P[Fs ≤ -6.126] = 0.0029*	P[rg ≤ 0.0061] = 0.0111^{+}	P[R2 ≤ 0.0944] = 0.1517

Total overall *T. pinchaque* sample (constant population)

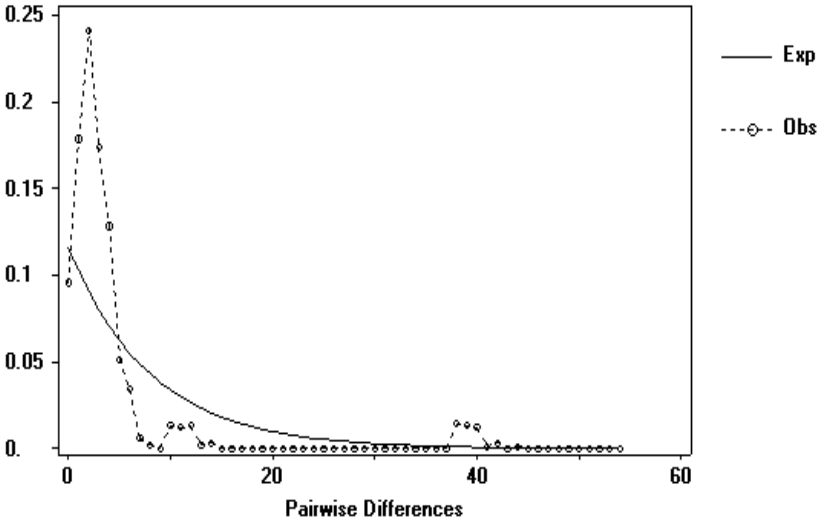

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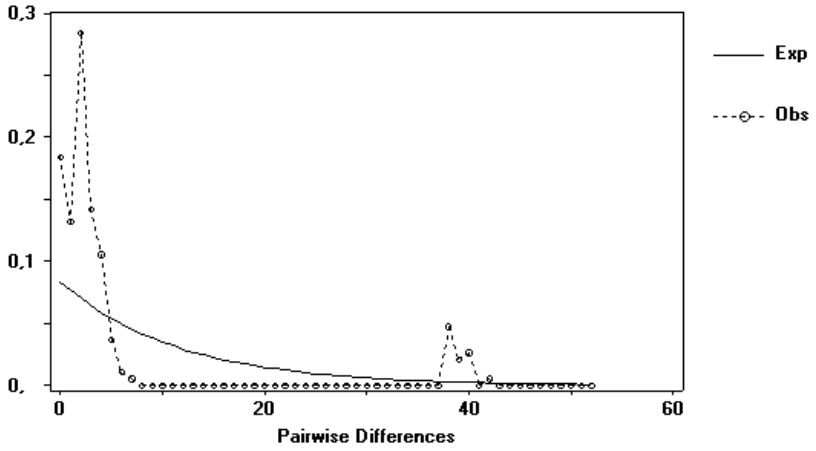
Figure 8. (Continued).

Colombian *T. pinchaque* sample (constant population)



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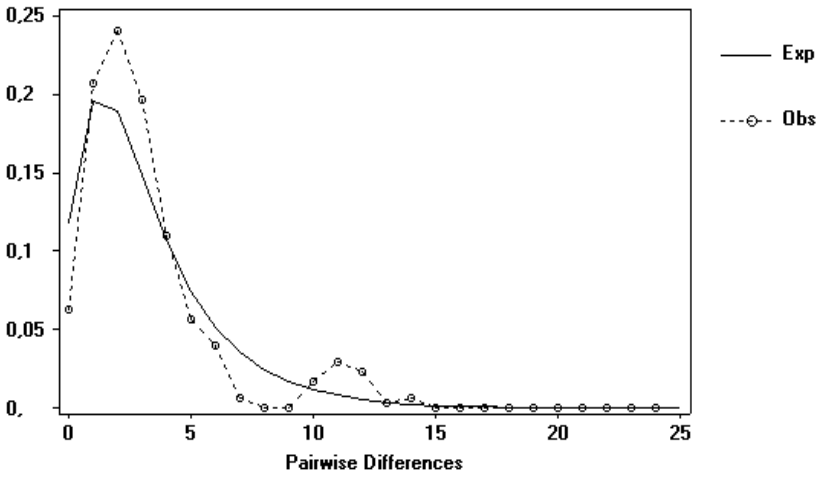
Ecuadorian *T. pinchaque* sample (population expansion)



C

Figure 8. Historical demographic analyses by means of the mismatch distribution procedure (pairwise sequence differences) for the mitochondrial DNA studied in *Tapirus pinchaque.* These analyses were applied to: Overall *Tapirus pinchaque* sample (A); Colombian mountain tapir sample (B); Ecuadorian mountain tapir sample (C).

Table 5. Demographic statistics applied to the overall *Tapirus terrestris* sample studied, to the haplogroups, to the population separated by the Amazon River and to "*T. kabomani*". * P < 0.05; ** P < 0.01, significant population expansions

	Tajima D	Fu & Li D*	Fu & Li F*	Fu's Fs	raggedness rg	R2
Overall *Tapirus terrestris* sample	P[D ≤ -1.642] = 0.018*	P[D* ≤ -4.00] = 0.004**	P[F* ≤ -3.59] = 0.004**	P[Fs ≤ -34.02] = 0.000**	P[rg ≤ 0.0035] = 0.0009**	P[R2 ≤ 0.0453] = 0.031*
Haplogroups						
Amazon I	P[D ≤ -0.909] = 0.176	P[D* ≤ -1.563] = 0.089	P[F* ≤ -1.611] = 0.101	P[Fs ≤ -4.517] = 0.008**	P[rg ≤ 0.051] = 0.145	P[R2 ≤ 0.089] = 0.003**
Amazon II	P[D ≤ -1.639] = 0.046*	P[D* ≤ -3.307] = 0.002**	P[F* ≤ -3.244] = 0.003**	P[Fs ≤ -9.990] = 0.000**	P[rg ≤ 0.051] = 0.346	P[R2 ≤ 0.088] = 0.256
Amazon III	P[D ≤ -1.818] = 0.015*	P[D* ≤ -3.152] = 0.005**	P[F* ≤ -3.198] = 0.013*	P[Fs ≤ -9.691] = 0.001**	P[rg ≤ 0.050] = 0.360	P[R2 ≤ 0.089] = 0.235
North	P[D ≤ -1.738] = 0.028*	P[D* ≤ -3.242] = 0.006**	P[F* ≤ -3.242] = 0.010*	P[Fs ≤ -6.728] = 0.008**	P[rg ≤ 0.051] = 0.422	P[R2 ≤ 0.091] = 0.202
South	P[D ≤ -1.714] = 0.033*	P[D* ≤ -1.687] = 0.050*	P[F* ≤ -1.954] = 0.041*	P[Fs ≤ -2.965] = 0.047*	P[rg ≤ 0.060] = 0.204	P[R2 ≤ 0.049] = 0.019*
Amazon River as a barrier						
North to the Amazon River	P[D ≤ -1.215] = 0.095	P[D* ≤ -2.489] = 0.020*	P[F* ≤ -2.345] = 0.026*	P[Fs ≤ -30.424] = 0.000**	P[rg ≤ 0.006] = 0.033*	P[R2 ≤ 0.060] = 0.112
Southern to the Amazon River	P[D ≤ -1.322] = 0.042*	P[D* ≤ -2.872] = 0.013*	P[F* ≤ -2.769] = 0.012*	P[Fs ≤ -6.695] = 0.024*	P[rg ≤ 0.006] = 0.003**	P[R2 ≤ 0.060] = 0.025*
"*T. kabomani*"	P[D ≤ 0.30550] = 0.6511	P[D* ≤ 0.311] = 0.6404	P[F* ≤ 0.3259] = 0.6397	P[Fs ≤ 1.2411] = 0.6601	P[rg ≤ 0.01532] = 0.0000**	P[R2 ≤ 0.079401] = 0.0024**

The mismatch distribution and the six demographic tests carried out showed a clear population expansion for the overall *T. terrestris* sample studied (Figure 9 and Table 5). All six tests were highly significant. There was clear evidence of population expansion in different haplogroups. For Amazon I, the mismatch distribution and two out of six tests were significant.

This was the haplogroup where there was less evidence of demographic change. For Amazon II, III and North, the mismatch distribution and four out of six tests were significant. For South, the mismatch distribution and five out of six tests were significant. Thus, we determined noteworthy evidence of population expansion within each haplotype lineage as well as for the total *T. terrestris* distribution. When we only considered two geographical groups (North and South of the Amazon River), there was also strong evidence of population expansion. For the Southern Amazon group, the mismatch distribution and six out of six tests were significant, whereas for the Northern Amazon group, the mismatch distribution and four out of six tests were significant.

Although, the sample of "*T. kabomani*" is modest, we also analyzed possible demographic changes in this taxon (Figure 9 and Table 5). The mismatch distribution and two out of six demographic tests showed evidence of population expansion such as was observed in *T. terrestris*, especially in the Amazon I haplogroup.

Thus, "*T. kabomani*" should be a dynamic demographic extension of *T. terrestris*, characterized by historical population expansions (proof 10).

Overall *T. terrestris* sample (population expansion)

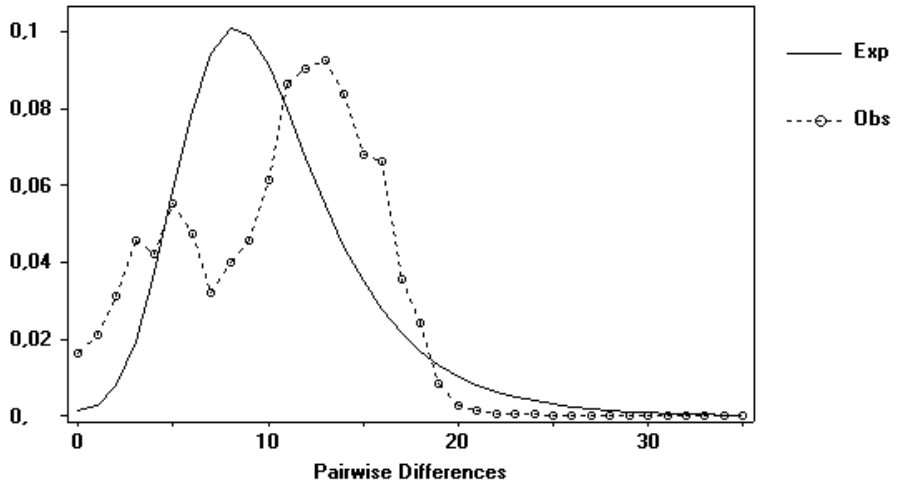


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Figure 9. (Continued).

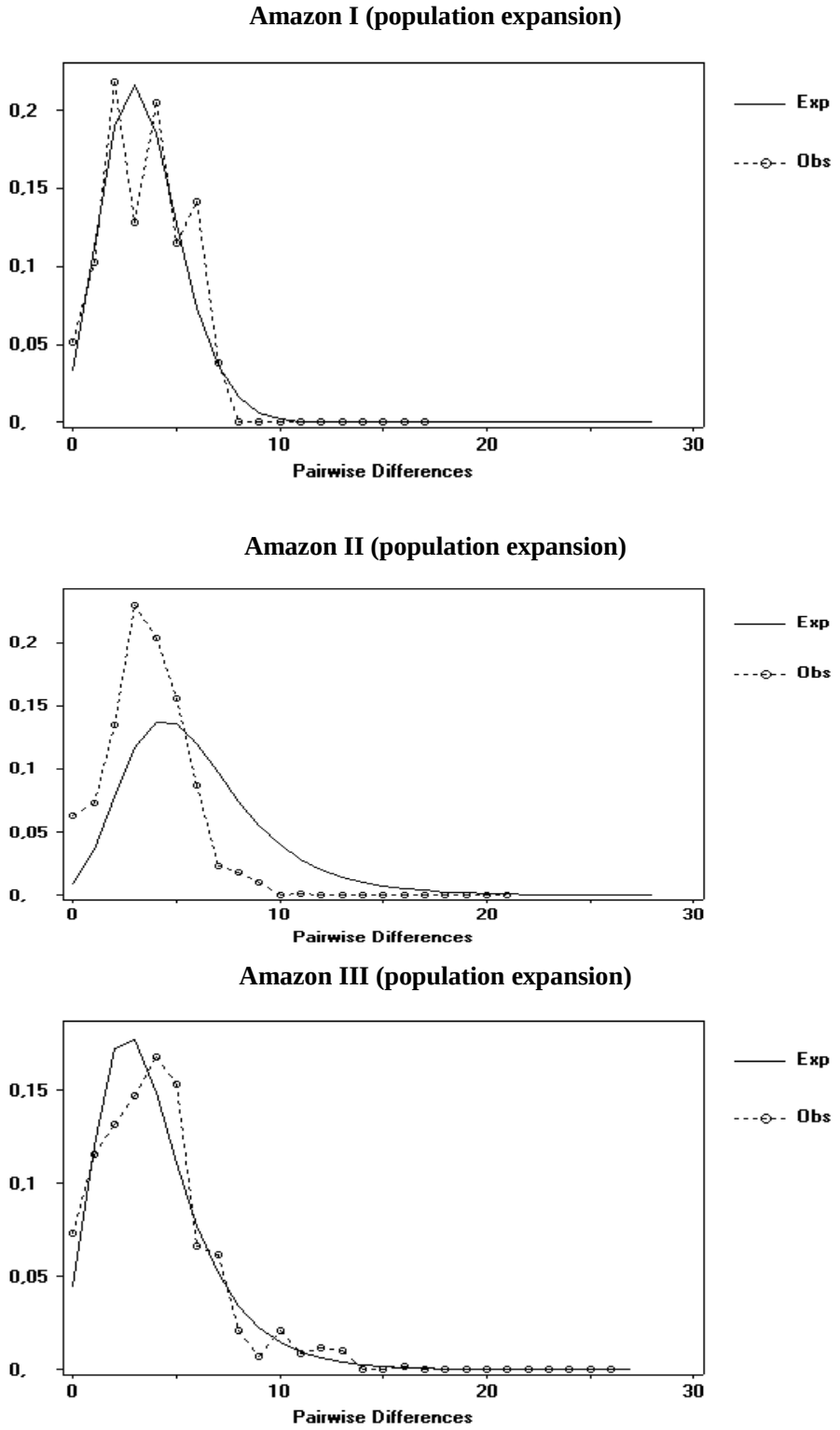
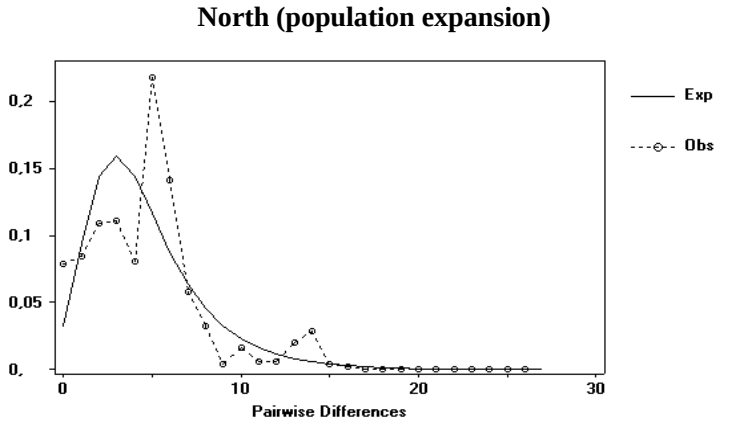
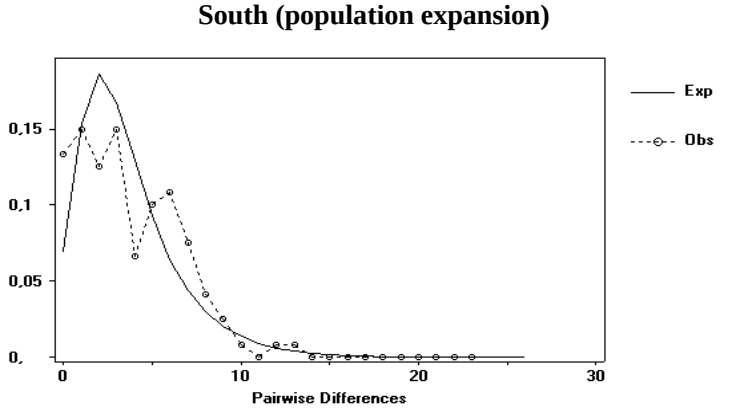


Figure 9. (Continued).





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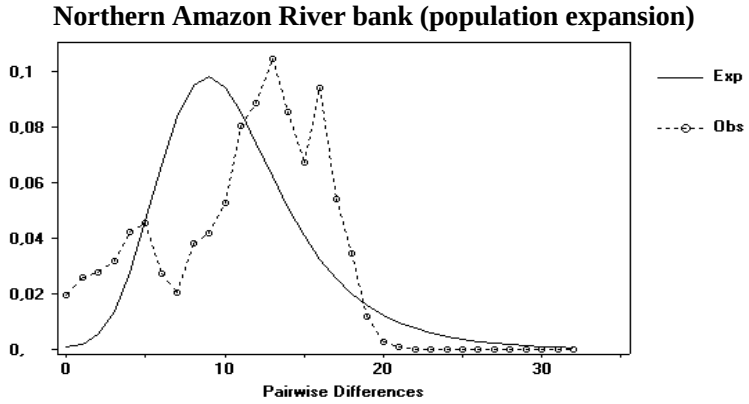
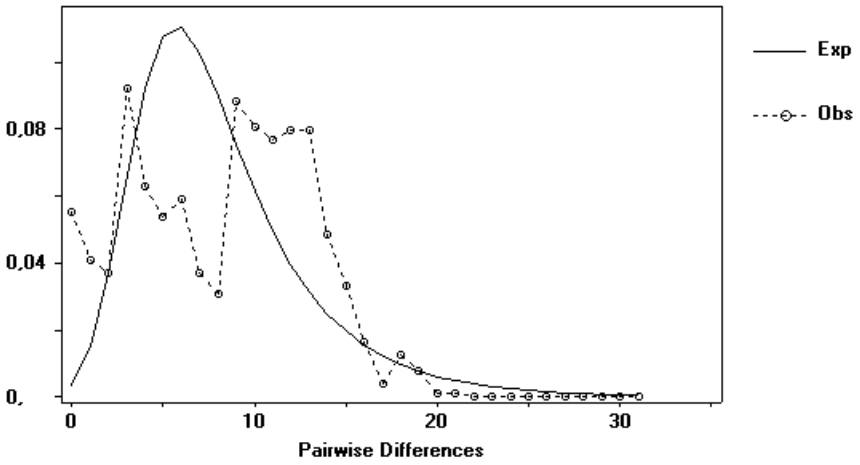


Figure 9. (Continued).

Southern Amazon River bank (population expansion)



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***"T. kabomani"* (population expansion)**

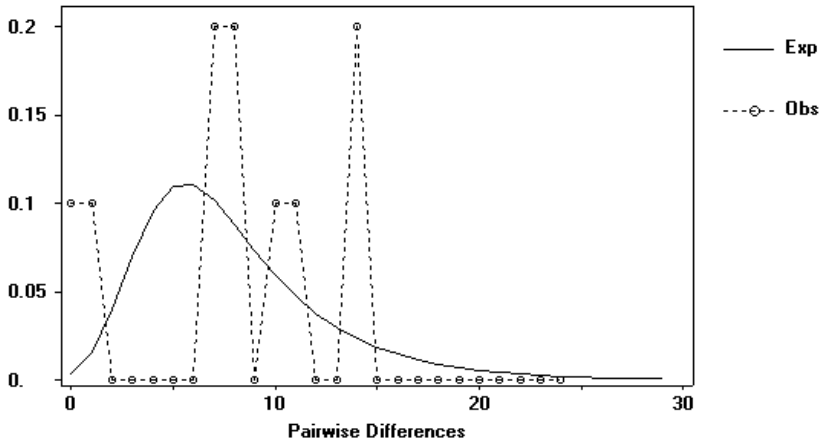

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Figure 9. Historical demographic analyses by means of the mismatch distribution procedure (pairwise sequence differences) for the mitochondrial DNA studied in *Tapirus terrestris* and "*T. kabomani*". These analyses were applied to: Total *T. terrestris* sample (A); Amazon I, Amazon II, Amazon III, North, and South (B); Northern bank of the Amazon River and Southern bank of the Amazon River (C); "*T. kabomani*" (D).

Spatial Structure

For *T. pinchaque*, the overall Mantel test between the log (genetic distance) and the log (geographic distance) showed r = -0.127 ($p < 0.691$ from 10,000 randomizations). Therefore, there is no measureable spatial genetic structure in *T. pinchaque*, at least, in the areas sampled in Colombia and Ecuador for mitochondrial DNA. Similarly, for *T. terrestris*, the Mantel test

did not detect any significantly positive correlation between the genetic distances and the geographical distances among the exemplars sampled at the global level, within different haplogroups and within certain geographical regions at the *Cyt-b* gene. Some correlations were significant but negative (for the global tapir population studied, r = -0.169, p = 0.001; Amazon I, r = -0.439, p = 0.001; Amazon II, r = -0.213, p = 0.001; Amazon III, r = -0.107, p = 0.023; North, r = 0.007, p = 0.439; South, r = -0.525, p = 0.001; total Colombia, r = -0.070, p = 0.007; Northern Colombia-Venezuela, r =-0.134, p = 0.001). Therefore, no isolation by distance patterns were found in any of the *T. terrestris* groups analyzed. Nevertheless, for *T. terrestris*, an AIDA analysis for the entire Amazon basin showed the 1 DC (0-350 km) to be significantly positive. The 4 DC (1,150-1,350 km) had a significantly negative autocorrelation and again a significantly positive autocorrelation at 6 DC (1,500-1,700 km). This correlogram showed the typical resemblance of a double circular cline (Sokal and Oden, 1978), which implied a complex pattern of colonization of this area by the lowland tapir.

In contrast, the *T. bairdii*'s haplotypes showed a very significant spatial genetic trend (r = 0.819, approximate Mantel t-test = 3.611, p = 0.0002; out of 5000 random permutations, 5000 were < Z, 0 = Z and 0 > Z; one-tail probability p = 0.0004), an effect of isolation by distance.

DISCUSSION

Genetic Conservation of the Latin American Tapirs

Clearly, *T. terrestris* had the highest gene diversity level of all tapir species. This finding agrees quite well with the fact that the Latin American *Tapirus* species had the widest geographical distribution and therefore the highest potentially effective numbers. Curiously, *T. pinchaque*, a species with a very restrictive geographic distribution and a small population size, presented a gene diversity that was more than three times higher than that of *T. bairdii*. The surprise is due to the fact that *T. pinchaque*, may be on the brink of extinction (Ashley et al., 1996) whereas *T. bairdii*, has historically occupied a distribution from Southern Mexico to the Pacific area of Colombia and maybe Ecuador (Tirira, 2011). Thus, although *T. pinchaque* has a very small census population size and a very restrictive geographical distribution, within disturbed and fragmented ecosystems, the species is not impoverished from a genetics point of view. This is good news from conservation perspective.

The geographical distribution of *T. pinchaque* is still not completely known and there is disagreement over its distribution within the scientific literature. In Colombia, populations of the mountain tapirs are well described in the Central and Eastern Cordilleras (Lizcano et al., 2002; Cavelier et al., 2010). Lizcano et al., (2002) estimated around 2,500 mountain tapirs in Colombia with around 1,874 animals in seven Colombian National Parks. However, Cavelier et al., (2010) estimated a minimum of 543 specimens and a maximum of 579 animals in the eight Colombian National Parks where they considered that this species still lives. However, the presence of the mountain tapir in the Western Colombian Cordillera is disputed. Acosta et al., (1996) and Downer (1997) recorded locations in this Cordillera where the species could have lived in the past. Sarriá (1993) recorded comments of staff working at Farallones de Cali National Park who observed *T. pinchaque* near Alto de Pance and Alto del Hambre (3,730 masl). Another possible record of the presence of *T. pinchaque* within the Western Cordillera

was made by De Wilde (1994), who wrote about footprints in the Caramanta Cerro between the Departments of Antioquia and Risaralda. However, Lizcano et al., (2002) suggested that this species does not inhabit the Western Cordillera. Furthermore, Lizcano and Cavelier (2000) considered that such records within the Western Cordillera may indicate the Baird´s tapir (*T. bairdii*) and not *T. pinchaque*. The Baird's tapir could also live within the montane forest of Central America, up to 3,000 masl. However, Arias-Alzate et al., (2010) recently reported the existence of a skull of a *T. pinchaque* in a Medellín museum sampled in 1911 from Páramo de Frontino in the Antioquia Department of Western Cordillera. This provides evidence of *T. pinchaque* living within the northern area of the Western Cordillera.

Lizcano et al., (2002) recorded the presence of the mountain tapir within the Central Cordillera from Los Nevados National Park (Caldas and Risaralda Departments) to the South. They also noted the mountain tapir within the Eastern Cordillera from Páramo de Sumapaz to the South. However, some populations of mountain tapir have been recently detected north of Páramo de Sumapaz within the Eastern Cordillera, including at Chingaza National Park and 12 other northern localities. Páramo de Pisba may be one of these sites (Montenegro, 2002). The current absence of mountain tapirs from Northern Central and Eastern Cordilleras could be the result of hunting activity (Lizcano et al., 2002). The extreme decline of the female population we detected for the Colombian *T. pinchaque* population at the genetic level with a Bayesian sky plot analysis (Ruiz-García et al., 2015b) correlated quite well with the fact of an extreme reduction in the potential habitat space of the Andean tapirs. Cavelier et al., (2010) showed that the current distribution for Andean tapirs covers 31,400 km^2, compared to 205,000 km^2 in the past. Similarly, the trend in distribution size for tapirs in Colombia is much lower today (14,385 km^2) than in the past (74,556 km^2). That is, the current value corresponds to 19% of the past distribution. The area where the species occurs is actually a collection of 35 forest patches. Most of the fragments are small and only four are larger than 1,000 km^2 (Lizcano et al., 2002). These authors showed that only five to six fragments have the minimum necessary size (826 km^2) to maintain at least 150 individuals, the estimated number to maintain a viable population in the short term. Thus, the situation of *T. pinchaque* is critical in Colombia.

In Ecuador, the areas with the highest *T. pinchaque* population sizes are the Cayambe-Coca National Park (Northern Eastern Cordillera) and the Sangay National Park (Central Eastern Cordillera). There are other areas of Ecuador, such as the Reserva Ecologica El Ángel (Carchi Province), Cajas National Park-Bosque Protector Mazán (Azuay Province) and the Condor Cordillera, where the mountain tapirs have lived but where there are no records of them in the last few decades (Downer, 1997; Tirira and Castellanos, 2001).

Cavelier et al., (2010) estimated a population size of 543-579 individuals for Colombia and 983-1,047 individuals for Ecuador. However, the gene diversity of *T. pinchaque* in Colombia is higher than in Ecuador. Dobzhansky (1971) showed that ancestral populations retain more elevated gene diversities than do derived populations. This may support that the original *T. pinchaque* population inhabited the Colombian Andean Mountains and later it expanded toward the southern mountains (Ecuador today). Our analyses detected the population expansion of the Ecuadorian population. However, more recently, the Colombian population decreased due to hunting and habitat fragmentation (high population decrease in the last 5,000 YA; Ruiz-García et al., 2015b). The Ecuadorian population has been less affected by human activity. Thus, the Colombian population could be in a more dangerous situation than the Ecuadorian population. Another *T. pinchaque* population in critical

situation should be the Peruvian population. Cavelier et al., (2010) only estimated between 41 to 43 mountain tapirs in the Peruvian protected Tabaconas Namballe National Sanctuary. Downer's (1997) work suggests the possibility of mountain tapirs inhabiting Eastern Tapal in the Cajamarca Province, south of the Jaén Province, and at the Piura Province. The Peruvian population needs a molecular population analysis.

The gene diversity levels of *T. terrestris* are the highest estimated for the Latin America tapirs and this species seems to not be significantly endangered from a genetics point of view. Also, the major part of the haplogroups showed historical population expansions. However, *T. terrestris* is a species threatened due to the subsistence hunting carried out by the Indian and "colonos" communities in South America. Another threat comes from the deforestation of the Neotropical forests due to extensive agriculture and ranching (Constantino et al., 2006). In many areas of South America, this species is more targeted than other animals by hunters. Here we provide three examples of hunting: 1- Our first example is the case of 22 Indian Izoceño-Guaraní communities in the "chaqueña" region of Bolivia, who intensively hunt tapirs (Barrientos and Cuéllar, 2003); 2-A second example is the case reported by Romero et al., (2013) in the community of Caura (Guarataro), along the Southern Orinoco River in Venezuela. There humans consume 40 % of their proteins from hunted animals. Of the 196.6 g of proteins consumed by a human per week, 77 g originated from wild hunts and of these, 14 were from tapirs. Out of 184 tons of wild meat consumed in a year, 44 tons were from tapirs. This means that in one year this community hunted 274 tapirs. 3- Third, it is clear that the tapir provided the most meat in the Peruvian rainforest compared to all other mammals, but unfortunately the hunting model used is non-sustainable. Bodmer et al., (1997a) calculated the percentage of production taken by hunters in the Pacaya-Samiria National Park within the Peruvian Amazon. For example, the hunted red deer (*Mazama amaricana*), collared peccary (*Pecary tajacu*) and white lipped peccary (*Tayassu pecari*) yielded values of 6.8 %, 4.3 % and 19.8 %, respectively. The authors considered that a value higher than 20 % could be dangerous to the continued subsistence of a population. The value for the lowland tapir in that Peruvian area was 166 %, which showed that the probability of subsistence of that population was completely un-sustainable. Also, Bodmer et al., (1997b) showed that in one year for three areas of the Pacaya-Samiria National Park, 53 *Tayassu pecari*, 12 *Pecari tajacu*, 13 *Mazama Americana* and 20 *Tapirus terrestris* were killed. But, tapir species have the highest biomass (4,000 kg) of all mammals, making up 48 % of the ungulate biomass extracted and 35 % of the total mammal biomass extracted. Thus, tapirs are critically affected by hunting. Furthermore, this species has a long gestation period of around 13 months, only producing one offspring by litter (Padilla and Dowler, 1994). Tapirs reach sexual maturity at two years of age (Yamini and Schillhorn, 1988), and females only have one offspring every three years. Tapirs also have a very low population density (Bodmer and Brooks, 1997; Bodmer et al., 2000). Bodmer and Brooks (1997) reported an overall density of 0.4 ind/km^2 for the Peruvian Amazon forests, while Ayala (2003) found a value of 0.5 ind/km^2 for the Bolivian Chaco. Scientists have offered a range of estimations from 0.028 ind/km^2 in the Northern Colombian Amazon (Chamorro-Rengifo and Cubillos-Rodriguez, 2007) to 1.2-1.6 ind/km^2 in the Chiquitanian forest of Bolivia (Arispe et al., 2007) and Brazil (Schaller, 1983). For this reason, the conservation of tapirs is crucial within Neotropical environments.

The reduction of the lowland tapir populations is observable because of its markedly reduced distribution. One example of this is the putative subspecies *T. t. colombianus*,that has been extirpated from the lowlands of the Caribe region as well as from the Andean valleys of

Colombia. This putative subspecies has been isolated in some localities of the Sierra Nevada de Santa Marta (Constantino et al., 2006). *T. terrestris* is classified in the Appendix II of CITES and the IUCN (2003) classified it as Vulnerable. Also, the subspecies, *T. t. colombianus*, is considered to be Critically Threatened (IUCN, 2004). In northern Argentina, the tapirr distribution range has been reduced by 46 % in the last century by hunting and habitat destruction (Chalukian et al., 2013).

Thoisy et al., (2010) affirmed that the Amazon River acted as a barrier to gene flow in *T. terrestris*. Our results showed that the Amazon River was only a partial barrier for haplotype dispersion for *T. terrestris*. An interesting additional comment related with this is that the jaguar corridor initiative proposed by Rabinowitz and Zeller (2010) and Zeller et al., (2013) could be very useful for the lowland tapirs too. The jaguar conservation units could be very similar and overlap the region with the largest tapir populations (they are the largest carnivore and herbivore mammals in South America). The 182 jaguar corridors proposed by these authors (2.6 million km^2) could also be useful in connecting tapir populations especially in the northern and southern geographical areas of South America where the habitat destruction carried out by humans is more intense.

The case of *T. bairdii* seems to be even more dramatic. Although its geographic distribution is wide, it has been dramatically reduced and fragmented in the last two centuries and today no more than 6,000 individuals are left in the wild (Ashley et al., 1996). Its mitochondrial genetic diversity levels were extremely low compared with other Neotropical tapirs. This could mean that this species suffered from a bottleneck and/or the gene drift has been more intense on this species by natural or human constrictions. Indeed, this species has intensely declined in the last century by habitat destruction and hunting and has been extinct in El Salvador and in a major fraction of its original distribution range in Colombian and probably completely extinct in Ecuador (Bodmer and Brooks, 1997).

Systematics of the Neotropical Tapirs and the Case of the Alleged "*T. Kabomani*"

All the molecular studies on tapirs, with the exception of that of Couzzol et al., (2013) and one of the analyses of Thoisy et al., (2010), determined the split between *T. terrestris* and *T. pinchaque* to have occurred during the last Pliocene or at the beginning of the Pleistocene. Ashley et al., (1996), analyzed the *COII* gene and determined the temporal split between the ancestors of *T. terrestris* and *T. pinchaque* to be around 3 MYA. The authors concluded that this coincided with the disappearance of the Bolivar Trough and the apparition of the modern Panamanian isthmus. Norman and Ashley (2000) added more Perissodactyla species and other mitochondrial gene (*12S rRNA*) and estimated new temporal splits. The *COII* and *12SrRNA* genes supported divergence times between *T. terrestris* and *T. pinchaque* of 2.5-2.7 MYA and 1.5-1.6 MYA, respectively. More recently, Ruiz-García et al., (2012), analyzed the *Cyt-b* gene and showed a Bayesian tree where the ancestors of *T. terrestris* and *T. pinchaque* diverged around 3.8 MYA (95 % High Posterior Density, HPD: 2.1-4.7 MYA). When a ρ statistic was used on a MNJ network, the two most frequent *T. terrestris*'s haplotypes diverged from the main *T. pinchaque*'s haplotype around 1.58 ± 0.30 MYA and 1.53 ± 0.34 MYA, respectively. Ruiz-García et al., (2015a) showed a Bayesian tree that supported a

temporal split of 3.33 MYA between both *T. pinchaque* and *T. terrestris*. Also, Ruiz-García et al., (2015b) performed a mitogenomic study of *T. pinchaque* and determined the temporal split between the ancestors of *T. terrestris* and *T. pinchaque* to oscillate from 7.42 to 3.27 MYA (95 % HPD) in a Bayesian tree. In that study also, with the ρ statistic from the MNJ analysis, and with no priors from other paleontological or molecular studies, assuming that *T. pinchaque* was an ancestral taxon with regard to *T. terrestris* (the Hershkovitz, 1954's and Haffer 1970's hypotheses), a split occurred between the taxa around 2.47 $\pm$ 0.42 MYA. In contrast, if *T. terrestris* is older than *T. pinchaque,* (Ruiz-García et al., 2012's hypothesis), the temporal divergence between them would have occurred around 3.59 $\pm$ 0.79 MYA. Our current work suggests that the split between species occurred 3.4 MYA.

These temporal estimates absolutely disagree with what Cozzuol et al., (2013) claimed. They reported a temporal split within the clade "*T. kabomani"-T. pinchaque- T. terrestris* ranging from 0.65 to 0.29 MYA (95 % HPD, with the split between *T. pinchaque* and *T. terrestris* around 0.1-0.3 MYA following these authors). In another approach, Thoisy et al., (2010), estimated the divergence between *T. terrestris* and *T. pinchaque* to have occurred around 0.33 MYA.

Nevertheless, we are more confident that the first estimates are more accurate than the second for three main reasons. (1) In the current work we used more individuals of all the Latin American tapir taxa and more genes (better gene diversity estimations in each taxa) than the Thoisy et al., (2010)' work. However, we used a mutation rate, in our MJN analysis that was close to the second mutation rate used by Thoisy et al., (2010). They obtained a very recent split between *T. pinchaque* and *T. terrestris* (0.33 MYA). The temporal split estimates provided by Ruiz-García et al., (2015a,b), ranged from 7.4 to 2.5 MYA. They were obtained without any prior or constriction made by the researchers on divergence times or mutation rates. Our current estimate of 3.4 MYA is within of the quoted range. (2) The extremely reduced divergence time put forth by Cozzuol et al., (2013) for the split among "*T. kabomani"-T. pinchaque- T. terrestris*, and which influences the appearance of "*T. kabomani*", is easily explained by a temporal constriction imposed by these authors in their data. They applied a constriction for the mitochondrial haplotype diversification in *T. terrestris* of 0.13 $\pm$ 0.1MYA because they affirmed that the oldest fossil records of *T. terrestris* date back to the beginning of the fourth Pleistocene glaciation (0.13 MYA). This paleontological constriction helps to explain the difference in temporal divergence between South American tapir species noted by Cozzuol et al. (2013) relative to other studies. These authors claimed that no clear *T. terrestris* fossil records dating older than 130,000 YA have been located, following Tonni (1992). He described a *T. terrestris's* mandible from the Lujanense age (upper Pleistocene, 130,000 YA) that he collected from the Colon Department at the Entre Rios Province (Argentina). Also, Noriega et al., (2004) and Ferrero et al., (2007), found fossil fragments of *T.* cf. *terrestris* corresponding to the El Palmar Formation at the El Boyero locality (upper Pleistocene) at the Entre Rio Province (Argentina). But, this does not mean that they don't exist, simply that they have yet to be found. Recently, Holanda and Rincón (2012) reported two tapir remains in Venezuela. One of them was classified as *T. terrestris* from the Zumbador Cave from the upper Pleistocene, while the second fossil from El Breal de Orocual was classified as *Tapirus sp.* It's unclear if this last fossil was from the Pliocene or from the early Pleistocene. For example, if this last remain is classified as *T. terrestris*, the Cozzuol et al., (2013)'s constriction doesn't make sense and therefore the divergence within the clade "*T. kabomani"-T. pinchaque- T. terrestris* should be considerably

older. Indeed, we believe that some of the oldest South American tapir fossil remains classified as *Tapirus sp* could belong to *T. terrestris*, especially if a population view is adopted more than a typological one, which is more typical of paleontologists. Hulbert et al., (2009) showed that the discovery of 75 individuals of *T. polkensis* in the Gray Fossil Site in Eastern Tennessee indicated a unique species. However, it had considerable intraspecific variation in the development of the sagittal crest, outline shape of the nasals and the number and relative strength of lingual cusps on the P1. These authors concluded that if these fossil remains had been found in diverse geographical areas, they would have been considered different species. Similarly, Perini et al., (2011) demonstrated that the lower molariform teeth size and proportions, used by many authors to define different tapir fossil species, are unreliable because they have great population variability. Thus, some early and middle Pleistocene tapir fossils should be re-assigned and some of them could be un-differentiated from *T. terrestris.* The findings of Perini et al., (2011) don't support the conclusion that all of these *Tapirus* fossils are *T. terrestris*, they simply indicate that some are not, but that others could be. Indeed, in our population criteria and in a context of anagenesis, or phyletic evolution, some fossil of tapirs, like *T. rondoniensis*, *T. cristatellus* and, even, *T. mesopotamicus* could be interpreted as *T. terrestris* individuals with some small morphological differentiated traits. These traits would probably be due to the exposure to different environmental conditions in each moment and in each Pleistocene refugium as well as due to phenotypic plasticity. Another controversial question between genetics and paleontological findings in regard to tapirs is the fact that several authors, from a paleontological point of view, claimed that the South American tapirs do not form a monophyletic group (Holanda and Ferrero, 2013). These authors maintain that *T. pinchaque, T. terrestris, T. mesopotamicus, T. rondoniensis* and *T. cristatellus* are paraphyletic with evolution *in situ* in South America—deriving from a similar form such as *T. webbi* from North America during the Miocene. They also claimed that another dispersal event occurred from South America to North America by means of a form similar to *T. cristatellus*, which gave origin to the derived forms of North America. This contrasts with the opinion of other authors such as Hulbert and Wallace (2005) and Hulbert (2010), who maintained that the North American forms were more primitive. From a molecular genetics perspective, Ruiz-Garcia et al., (2012), and the current work, found that the two extant tapir species in South America only belonged to a unique migration wave, whilst *T. bairdii* belonged to another different molecular group, older than the first and probably more related to the fossil tapirs from North America. Thus, the molecular results seem to agree better with the second paleontological hypothesis than with the first one. 3- Within the Perissodactyla order, there are no cases of an appearance of new species in the last 0.1-0.3 MYA. In Equidae, Xu (1996), took into account all of the mitochondrial DNA and estimated the initial splitting within *Equus* to have occurred approximately 9 MYA. Tougard et al., (2001), by means of the *12S rRNA* and *Cyt-b* genes, determined that time split to be 12.2 $\pm$ 2.2 MYA. Recently, Orlando et al., (2013), sequencing more than 5,000 genes, determined that all contemporary horses, zebras and donkeys originated 4.0–4.5 MYA. Furthermore, they determined the divergence time between populations of Przewalski's and domestic horses to be approximately 0.38-0.72 MYA (different horse subspecies). In Rhinocerotidae, the split between the current species is estimated to be around 26 MYA (isoenzymes; Merenlender et al., 1989) and 21.5 MYA (*12S rRNA* and *16S rRNA* genes; Morales and Melnick, 1994). The molecular temporal divergence between the two Asian rhinoceros genera was around 25.9 $\pm$ 1.9 MYA (Tougard et al., 2001).

Their paleontological estimates oscillated from 16 to 23 MYA (Carroll, 1988). The molecular split for the two Asian rhinoceros species of the genus *Rhinoceros* (*R. sondaicus* and *R. unicornis*) was molecularly estimated to have occurred around 11.7 ± 1.9 MYA (Tougard et al., 2001). Their paleontological split has been estimated to have occurred 1.6-3.3 MYA (Carrol, 1988). The two traditional subspecies of the white rhinoceros (*Ceratotherium simum simum* and *C. simum cottoni*) have been estimated to diverge around 1 MYA (Groves et al., 2010). Henceforth, the claim by Cozzuol et al., (2013) of a divergence time of around 0.1-0.3 MYA between *T. terrestris* and *T. pinchaque* does not agree with that determined for other current Perissodactyla taxa.

With regard to the systematics of *T. pinchaque*, our genetics data make a remarkable contribution. Hershkovitz (1954) found a variable trait in the *T. pinchaque* dentition, as manifested by the first upper premolar, which is variable. Ecuadorian specimens show the simple condition, with the cinguloid shelf absent, whereas the first Colombian *T. pinchaque* skull examined shows the premolar as another *Tapirus* species. For this reason, he distinguished two subspecies (*T. p. pinchaque* and *T. p. leucogenys*) within the mountain tapir. However, our molecular results showed that Colombian and Ecuadorian specimens were mixed independently of their geographical origins disagreeing with the fact that they are different subspecies.

With regard to the systematics of *T. terrestris*, the existence of the six mitochondrial haplotype lineages did not agree at all with the four morphological and geographical subspecies determined by Cabrera (1961) and Hershkovitz (1954). That is, there was no correspondence among the mitochondrial haplogroups and the morphological subspecies. In the geographical area of *T. t. aenigmaticus*, we found the six different haplogroups detected in this study. In the wide distribution of *T. t. terrestris*, four haplogroups were found (Amazon II and III, North and South). Thus, there was no correspondence among the subspecies *T. t. anigmaticus* and *T. t. terrestris* and the mitochondrial lineages herein showed. We tentatively negate the validity of these two subspecies. In the territory of *T. t. colombianus*, two haplogroups were detected, Amazonian II and North. Theoretically, the animals sampled in the Colombian Departments of Antioquia, Córdoba and Magdalena (Sierra Nevada de Santa Marta) and at the Zulia (Maracaibo Lake) in Venezuela belonged to *T. t. colombianus*. However, one animal from the Antioquia Department belonged to the Amazonian II haplogroup. Animals from Meta, Vichada and Guainia Departments (Colombian Eastern Llanos and Amazon), French Guyana, Madre de Dios River (southern Peruvian Amazon), Yavari River (Western Brazilian Amazon) and even animals from Northern Argentina shared haplotypes with the North haplogroup. Therefore, there is no linear correspondence between *T. t. colombianus* and the North haplogroup, which questions the reality of this subspecies. The case of these animals from Northern Argentina within this haplogroup is extremely strange. It could be that animals with the same haplotypes migrated from the upper part of the Western Amazon to the northern and southern distribution ranges. And, for this reason, both extreme geographical areas (Northern Colombia and Northern Argentina) share mitochondrial haplotypes. Another possibility, which must be remotely considered, is the fact that during the XIX century and the beginning of the XX century, people imported tapirs from northern areas of South America and liberated them to Northern Argentina for game hunting purposes (Martínez, personal communication). This could explain the presence of haplotypes of the North haplogroup in Northern Argentina.

All the animals we sampled in Southern Brazil, Eastern Bolivia and Paraguay, which "a priori" belonged to *T. t. spegazzinni* by its geographical distribution and morphological features, belonged to the South haplogroup. Therefore all the southern animals belonged to a unique mitochondrial haplogroup, with the exception of the Argentinian individuals. In this case, it could be a linear correspondence between the South haplogroup and *T. t. spegazzinni*. However, some animals from the Central Brazilian Amazon and from the Colombian Amazon also showed haplotypes of this South haplogroup. It is also interesting to note that traditionally only *T. t. spegazzinni* has been cited in Bolivia (Anderson 1997). In Bolivia, 35 specimens have been located representing 24 localities (Salazar-Bravo et al. 2003). We found many tapirs of Bolivia belonging to the South haplogroup (related with *T. t. spegazzinii*), but we also determined exemplars of the Mamore River which belonged to the Amazonian II lineage typical of more Northern Amazon areas.

Due to the complex spatial distribution pattern of *T. terrestris* garnered through analysis of the *Cyt-b* gene it is impossible to assign tapirs with unknown origin and living in captivity to precise geographical locations. However, we can assign them to one of the six haplogroups we found. For instance, the five tapirs from the US zoos belonged to three out of the six haplogroups (Amazon I, III and South). Also, the exemplar sampled at the Barcelona Zoo belonged to the South haplogroup. Moreover, the animal of unknown origin we analyzed was assigned to the North haplogroup. It was a unique tooth that had no recorded origin. This complex spatial structure could complicate the application of some biological conservation concepts, such as ESUs (Evolutionary Significant Units) and MUs (Management Unit) (Moritz and Faith, 1998) to *T. terrestris*.

Our mitochondrial data totally disagree with the results of Cozzuol et al., (2013). They claimed that "*T. kabomani*" was a full species. We provide 10 population genetics proofs against this claim. All of our trees, as well as those from Ruiz-García et al., (2015a,b,c) showed that "*T. kabomani*" is a particular lineage within *T. terrestris*. In a mitogenomic study with 15 genes and more than 250 *T. terrestris* individuals, Ruiz-García et al. (2015c) showed reciprocal monophyly between *T. terrestris* and *T. pinchaque*. Also, "*T. kabomani*", together with the Amazon I haplogroup, were the first haplogroups to diverge within *T. terrestris*. In the current work, with three mitonchondrial genes, the genetic distances between *T. terrestris* and "*T. kabomani*" were always lower than the genetic distances obtained between *T. terrestris* and *T. pinchaque*.

Kartavtsev (2011) analyzed sequences of the *COI* gene from 20,731 vertebrate and invertebrate animal species. He estimated the average distance data for five different groups. He obtained 0.89% ± 0.16% for populations within species, 3.78% ± 1.18% for subspecies or semispecies, 11.06% ± 0.53% for species within a genus; 16.60% ± 0.69% for species from different genera within a family and 20.57% ± 0.40% for species from separate families within an order. For this gene, the genetic differentiation between *T. terrestris* and *"T. kabomani*" was only 0.5 %, clearly within the status of populations within species. Ascunce et al., (2003) and Collins and Dubach (2000) reported for Primates at the *COII* gene, an average genetic distance around 5.82% among species within a genus and around 2-4 % for subspecies. For this gene, the genetic differentiation between *T. terrestris* and *"T. kabomani*" was only 1.3 %, clearly within the status of populations within species. Bradley and Baker (2001) claimed, for the *Cyt-b* gene, that values < 2% would equal intra-specific variation, values between 2% and 11% would merit additional study, and values >11% would be indicative of specific recognition. For this marker, the genetic differentiation between *T.*

terrestris and *"T. kabomani*" was 1.8 %, clearly within the status of intra-specific variation. The surprising question is the small genetic distances between *T. pinchaque* and *T. terrestris*, because these taxa have traditionally been considered full species. Thus, considering molecular genetic distances, *T. pinchaque*, should be considered a sub-species of *T. terrestris*. However, we consider *T. pinchaque* a full species because no natural or captivity hybridization has been reported due to reproductive barriers via stasipatric speciation with chromosomal changes (White, 1968, 1978).

The inaccurate result obtained by Cozzuol et al., (2013) was probably due to the fact that these authors only analyzed five samples of *T. pinchaque* at the *Cyt-b* gene and only one sample at the *Cyt-b + COI + COII* genes. Indeed, they only analyzed three samples at the *Cyt-b* gene, because two *T. pinchaque* samples were repeated due to that the two samples of *T. pinchaque* that M. Ruiz-García donated to the Cozzuol's team were also donated by another scientist (that shared samples of the same animals with M. Ruiz-García) to B de Thoisy, who latter added his *T. pinchaque*' results to those of Cozzuol et al., (2013). The very small *T. pinchaque* sample used by Cozzuol et al., (2013) probably did not represent all of the mitochondrial gene diversity of *T. pinchaque.* This contrasts with the larger *T. terrestris* sample containing animals from a very wide geographical area and thus retaining a major fraction of the mitochondrial gene diversity of this last species, and as the genetic distances between both *T. terrestris* and *T. pinchaque* were very small, by chance the no representative mitochondrial gene diversity of *T. pinchaque* of the small sample of Cozzuol et al., (2013) was nested within the gene diversity of *T. terrestris.* Nevertheless, as soon as we enlarged the *T. pinchaque* sample, collected from a wider sampling area, this phenomena disappeared.

Cozzuol et al., (2013) also provided alleged evidence of morphological and morphometric differences of "*T. kabomani*" from the remaining living and fossil South American tapir species. In this work, we provide molecular proofs that do not support the highly speculative and inconclusive conclusions of Cozzuol et al., (2013) that favor "*T. kabomani.*" We also provide several comments on the alleged morphological differences between "*T. kabomani*" and *T. terrestris*. In a recent study, Ruiz-García et al., (2015c) analyzed several *T. terrestris* populations throughout Northern Colombia and different areas of the Amazon basin in Colombia, Peru, Brazil and Bolivia from a craniometric perspective (around 160 skulls). Most of these populations showed significant statistical differences regarding the two areas of the skull where Cozzuol et al., (2013) found the greater divergence of "*T. kabomani*" with regard to *T. terrestris* (the position of the frontal-parietal suture related with the beginning of the sagittal crest and frontals broad and more inflated behind the nasals in "*T. kabomani*"). The significant differences of these skull from those studied by Ruiz-García et al., (2015c) within diverse *T. terrestris* populations were related to different ontogenetic patterns within each population as well as to the age composition of each population. The statistical differences were extreme among some of the seven *T. terrestris* populations studied for different morphometric traits (1- the Napo River area in the Northern Peruvian Amazon, 2- Yavari River and neighborhoods in the Amazonian border of Colombia, Brazil and Peru, 3- Sierra Nevada de Santa Marta in Northern Colombia, 4- Meta, 5- Caqueta, and 6- Vichada departments, all three in Colombia, and 7- the Mamore River in the Bolivian Amazon). However, we don't claim that each one of these populations was a different tapir species, such as Cozzuol et al., (2013) claimed with *"T. kabomani"*.

Additionally, we show two other morphological proofs which don't support the claims of Cozzuol et al., (2013). For example, supposedly *T. kabomani,*" with its lower sagittal crest

and wider post-nasal exposition of frontals, is much smaller and has a darker color than the "typical" *T. terrestris*. In Figure 1A, we show a tapir captured in the Amacayacu River (Colombian Amazon) with a "*T. kabomani*" mitochondrial haplotype. We also show, in Figure 1B, a "typical" morphological *T. terrestris* individual from Tena, at the upper Napo River in Ecuador. But, it had a "*T. kabomani*" haplotype. Both animals exhibited different morphotypes to that claimed by Cozzuol et al., (2013) for "*T. kabomani*". For example, the Ecuadorian animal showed a considerable size and its color was brown. In contrast, as we show in Figure 10, in our travels to obtain tapir samples, we have found small-sized lowland tapirs. Figure 10A shows a pair of small-sized tapirs sampled in the Colombian Amazon, whereas Figure 10B shows a small tapir sampled in the Ecuadorian Amazon. We inspected the dentition of all three examplars and determined them to be adults because each had at least two erupted molar. Nevertheless, no one showed "*T. kabomani*" haplotypes. The Colombian exemplars belonged to the Amazon II and III haplogroups, while the Ecuadorian individual belonged to the Amazon I haplogroup. Indeed, these animals were of a size smaller than the two individuals in the photo published by Cozzuol et al., (2013) and that were defined as "*T. kabomani.*" Additionally, and mistakenly, they affirmed the existence of photos of "*T. kabomani*" from Colombia. This was a mistake because we provided the authors with two Colombian samples that they claimed to be "*T. kabomani.*" Furthermore, we did not provide any photos of these animals. Most likely, they never asked for the morphology of these exemplars to verify a link between these "*T. kabomani*" haplotypes and the size and dark coat color of these individuals. Complementary, we have observed some lowland tapir adults with small sizes and certain bones deformations as if they were affected by malnutrition or some kind of genetic syndrome (for instance, small head and body but large feet).

a

Figure 10. (Continued).

b

Figure 10. Some *T. terrestris* adult exemplars with very small sizes but without "*T. kabomani*" mitochondrial haplotypes. Two small adult individuals from Leticia, Colombian Amazon (A); one small adult individual from Macas, Ecuadorian Amazon sampled by the first author (B).

Dobzhansky (1937), in his seminal book for the Neodarwinism synthesis, showed that in evolution the approaches should be of a population nature rather than a typological one. It is quite understandable that a paleontologist (such as is the first author of Cozzuol et al., 2013), working with the scarce material that provides the fossil record on most of the occasions, keeps a typological morphology vision of the species concept. Indeed, many paleontologists only accept a cladogenetic mechanism of speciation following the principle of the punctuated equilibrium theory (Eldredge and Gould, 1972; Gould and Eldredge, 1993). This means that the punctuated equilibrium can become tautological because paleontologists define fossil species on the basis of morphological change. So, it is a trivial problem to observe a strong correlation between speciation and morphological changes. However, population geneticists and evolutionary biologists (including Darwin) know the existence of rapid morphological evolution in a hypothetical ancestral form without speciation (phyletic transformation or anagenesis). Therefore, the existence of a certain morphological and morphometric skull variability in the lowland tapirs did not mean the real existence of different species. Furthermore, the "*T. kabomani*" case is a current living case, and not a paleontological one. With a living species, there are many other aspects to be analyzed in addition to changes in morphology before concluding on the existence of a new species. And, changes in morphology are the only aspects that can be detected in the fossil record. This project and other studies show that the samples sizes and the interpretation of mitochondrial and craniometric data by Cozzuol et al., (2013) are insufficient to define a new tapir species in the Amazon. Additionally and unfortunately, Cozzuol et al., (2013) did not provide sufficient geographical (they contrarily argue that "*T. kabomani*" is simpatrically extended by all the

Amazon with *T. terrestris*), ecological, ethological or reproductive (post and/or prezygote) barrier information to claim to "*T. kabomani*" as a new full species. This does not mean that other tapir species do not exist, but that the provided data are insufficient and misunderstood. In what-ever-the-case, nuclear DNA and especially comprehensive karyological studies (due to the possibility of stasipatric speciation; White, 1968, 1978) should be carried out before claiming the existence of new species of tapirs.

The Impact of the Pleistocene Changes on the Phylogeography of *T. pinchaque* and *T. terrestris*

The temporal split between the ancestors of *T. terrestris* and *T. pinchaque* occurred around 3.4 MYA. And, the beginning of the *T. terrestris* mitochondrial diversification was around 3.1-3.3 MYA. These times match up well with the end of the Pliocene and the beginning of the Pleistocene (2.6-1.8 MYA; Van der Hammen, 1992), which were extremely cold. The average temperature descended 4 ºC $\pm$ 2 ºC and rainfall declined (500-1000 mm less than today) (Van der Hammen, 1992, 2001). The Pleistocene had 25 main glacial-interglacial cycles with climatic cycles changing every 20,000 Y for the first million years and thereafter every 100,000 Y. These climatic cycles had oscillations in temperature of 10 ºC at 2,500 m above sea level (masl). This first lowland tapir haplotype split (3.0-3.3 MYA) also coincided with the last formational phase of the Central Andes. The entire Andean chain between Cajamarca and Huancavelina was formed during this epoch by volcanic activity. The lagoons in Huancho (Northern Lima) and the bay in La Ventanilla Beach also formed during this time (León-Canales, 2007). The diverging process of the Amazon 0 haplogroup or "*T. kabomani*" and the haplotype diversification within *T. pinchaque* began around 1.3-1.2 MYA. This coincides with the Pre-Pastonian glacial period (1.3-0.8 MYA), which was extremely dry and cold. While the Buenos Aires province where mammalian fauna (*Categonus, Cyonasua, Clyomys*, etc) lived was basically tropical from 2-1.3 MYA, this climate later changed. This area was Patagonian Steppe-like around 1.3-0.8 MYA which agrees quite well with a dry and cold climate (Forasiepi et al., 2007). Examples of mammalian fauna living at this time were guanaco, *Lestodelpys* and *Lyncodon*. The diversification process within some of the *T. terrestris* haplogroups occurred around 0.5-0.3 MYA which showed that the same climatic events affected these lowland tapir haplogroups. This correlates to the beginning of the Kansas-Mindel glacial period, 0.4 MYA. Much of the haplotype proliferation we note in the haplogroups identified occurred around 0.2-0.06 MYA and in 0.03-0.01 MYA. This can be explained by the extreme climatic changes in these epochs. The last glacial-interglacial super-cycle began around 0.13-0.15 MYA (Würm. Wisconsin, Vistula, Gambliense). From 130,000 to 80,000 YA (Eemiense period), the climate was hot, the precipitation greater, and there were extensive swampy forests of *Aliso, Vallea* and *Weinmannia* (Van der Hammen, 1992). But, an extremely cold climate (upper Pleniglacial period) began around 70,000 YA which could have had a fragmentation effect on the lowland tapir haplogroups. From 30,000 YA to 10,000 YA there were many periods of extreme dry and cold conditions. For example from 30,000 YA to 23,000 YA, there was a very cold period in Europe named Dryas I. During this period, the Fuquene Lagoon near the current Bogota (Colombia) dried (29,000 YA; Van der Hammen, 1992) and Mac Neish (1979). This determined changes in the soil acidity and the types of pollen at the Pikimachay Cove found within Peru which are related to

the extreme cold 23,000 YA. Later, around 19,000-16,000 YA, the Last Glacial Maximum (LGM or Dryer II) occurred, which had the most extensive distribution of snow and ice in the Central Andes within the last 200,000 YA. Metivier (1998) estimated that about 18,000 YA, the glacial extension in North and Central Andes was around 371,306 km^2, whereas it is currently around 3,220 km^2 (only 1% of the LGM). Also, there were intense cold periods from 14,000 YA to 10,000 YA (with alternating hot periods). Rodbell and Seltzer (2000) found moraines (the front of a glacial) up to 3,170 masl (12,000 YA) at the Cordillera Blanca (San Martin Department, Peru). Comparatively, today the glacial front is 4,600 masl. Also, Rodbell (1991) determined that the other areas of the Cordillera Blanca within the limits of the Departments of Huánuco and Ancash (17 glaciers) had snow lines at 4,200 masl, whereas today this limit is 500-900 m higher. Similarly, Seltzer (1987, 1990) determined the snow lines in the Huaytapallama, Junin and Huancavelina Departments (Peru) 10,950 YA to be 1,400 m lower than today. These climatic conditions could have caused the last lowland tapir haplotypes to diversify.

Several authors (Ab'Saber, 1982; Brown et al., 1974; Fjeldsä, 1994; Haffer, 1969, 1974, 1982, 1987, 1997, 2008; Prance, 1982, 1996; Prance and Lovejoy, 1985; Terborgh, 1992; Van der Hammen, 1975; Vanzolini, 1970, 1973, 1992; Vanzolini and Williams, 1970; Whitmore and Prance, 1987) claimed that these climatic events and subsequent dry/humid cycles created in the Amazon basin are the result of Milankovitch cycles. During the dry periods rainforest communities split into isolated refugia separated by savannah or arid pampas. This hypothesis was originally established for the Pleistocene, but later expanded to include the Miocene-Pliocene periods as well and it was named the Refugia Hypothesis. This hypothesis could explain the appearances of the different haplogroups within *T. terrestris*. During each dry period the haplogroups were formed, but later during the humid periods the tapirs migrated allowing its different haplogroups to diversify in different geographic areas. Along with this, the relationship between the original refugia areas was lost which helped create the geographical distribution of tapirs we see today. However, some Pleistocene refugia could have played an important role in the origins of the haplogroups. For example, the Amazon I and the "*T. kabomani*" haplogroups could have radiated from the Napo refugia (Northwestern Amazon), while the Amazon II and III haplogroups could have radiated from the Napo and Inambari refugia (Southwestern Amazon). The South haplogroup could have expanded from the Inambari or from the Rondonia refugia (between the Madeira and the Tapajos rivers), whilst the North haplogroup could have originated from the Bolivar refugia within the Guiana area. Another hypothesis, called the Recent Lake hypothesis (Klammer, 1984; Marroig and Cerqueira, 1997; Nores, 1999, 2004; Sombroek, 1966), could also support the formation of these lowland tapir haplogroups. This hypothesis claims that most of Amazonia was covered by a huge lake or lagoon during the Pliocene, and successively smaller portions of Amazonia were covered during a series of assumedly high sea level events during the Pleistocene. These created different islands and archipelagos within this Amazon lake. The different haplogroups could have been created on these islands. However, like the tapirs, who are exceptionally good swimmers, these haplogroups could have migrated throughout aquatic systems and expanded their original geographical areas. Therefore, both the Refugia and the Recent Lake hypotheses could support the existence of lowland tapir haplogroups. The significant and complex spatial structure found for the lowland tapirs in the Amazon basin by means of AIDA agrees quite well with both of these hypotheses.

Using Bayesian sky plot analyses, we determined the population changes over the last few thousand years for some of the lowland tapir haplogroups and geographical areas studied. Amazon I suffered a population decline 5,500 YA coinciding with one of the dry periods of the Holocene after the Optimum Climaticum (OP). Following Rothlisberger (1987), around 6,300 YA, there was a significant increase in temperature especially in Southern South America as well as in the Central Andes, evidenced by O_{18} levels in Huascaran snow. This dry period around 5,500 YA was detected in the Amazon, Caqueta and lower Magdalena River basins as well as in Andean lagoons in Colombia and Peru (Thompson et al., 1995; Van der Hammen & Cleef, 1992; Van der Hammen, 2001). However, in the last 1,400 years, this haplogroup again increased. Amazon II and III revealed a population decrease 75,000 YA coinciding with the ending of the Eemiense inter-glacial period and the beginning of the upper Pleniglacial period (Würm I; Van der Hammen, 1992). These two Amazon haplogroups began to again expand around 12,000 and 14,000 YA respectively. This could have been the result of finishing the coldest and driest period of the fourth Pleistocene glacial period, a period that is also related to the last major extinction period for mammals.

In contrast, North decreased 14,000 YA, agreeing with the massive extinction of mammals across the Earth including in South America. This corresponds with the Younger Dryas (Dryas III), typical of Northern Europe and Scandinavia (Clapperton, 1993). This means that the end of this extremely cold and dry period was not the same for all of South America and therefore the diverse haplogroups of tapirs were differentially affected. The Amazon's climate was not very affected by these drastic conditions—leading to an increase in some Amazon haplogrpups. However, in Northern South America, the Younger Dryas was more drastic and the North haplogroup was negatively affected. Nevertheless, this lineage increased around 3,000 YA, just when the average temperature reached a value similar to what it is today (Van der Hammer, 1992). Finally, South suffered a population declination around 6,000 YA coinciding with the drier period between 7,000-5,500 YA that we commented on above.

When the data were analyzed by geographical area, only one region showed an important population declination in the last 5,000 years. This was the lowland tapir population of Northern Colombia and Northwestern Venezuela. This strong population decrease, detected by genetic methods, agrees quite well with the situation of this population, which was considered Critically Threatened in 2004 by the IUCN (Constantitno et al., 2006).

In contrast to what was expected, in the Andes Mountains, the Pleistocene climatic changes did not create a phylogeographic structure in *T. pinchaque*. This is a conundrum because in other Andean mammals, such as the Pampas cat (*Leopardus pajeros*) (Cossíos et al., 2010; Ruiz-García et al., 2013), the Andean cat (*L. jacobita*) (Cossíos et al., 2012; Ruiz-García et al., 2013) or the spectacled bear (*Tremarctos ornatus*) (Ruiz-García, 2003, 2013; Ruiz-García et al., 2003, 2005), the phylogeographic structure for different kinds of molecular markers were significantly marked. This may support that the haplotype diversification within the current *T. pinchaque* occurred more recently than it did in *T. terrestris*. It's also possible that the gene flow capacity of *T. pinchaque* was relatively higher for this species in the Andean cordillera than for *T. terrestris* in the Amazon lowlands. The population expansion detected in the Ecuadorian *T. pinchaque* population could correlate with this last idea. If the mitochondrial diversification of *T. pinchaque* occurred much more recently than in *T. terrestris*, this could agree with an event of anagenesis, or phyletic evolution, with the original ancestor more related to *T. terrestris* than to *T. pinchaque*. *T. pinchaque* could have

appeared via adaptation and exploitation of a new habitat within the Andean Highlands from a lineage, or population, of *T. terrestris.* It could have also appeared via stasipatric speciation. However, all of our phylogenetic trees do not agree with this perspective.

To complement these new mitochondrial results future studies should include sequences of HLA markers, autosomic and sexual chromosome introns and other nuclear DNA genes. Also, future studies focus on sequencing of tapir fossil remains may be helpful in our understanding of the evolution of the current mega-herbivores of South America.

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REFERENCES

Ab'sáber, A. N. (1982). The paleoclimate and paleoecology of Brazilian Amazonia. In Prance, G. T. (Ed.). *Biological Diversification in the Tropics.* New York: Columbia University. Pp. 41-49.

Acosta, H., Cavelier, J., Londoño, S. (1996). Aportes al conocimiento de la biología de la danta de montaña, *Tapirus pinchaque* en los Andes Centrales de Colombia. *Biotropica* 28: 258-266.

Ashley, M. V., Norman, J. E. Stross, L. (1996). Phylogenetic analysis of the periossodactylan family Tapiridae using mitochondrial cytochrome c oxidase (COII) sequences. *Journal of Mammalian Evolution* 3: 315-326.

Anderson, S. (1997). Mammals of Bolivia, Taxonomy and Distribution. *Bulletin of the American Museum of Natural History* 231: 1-652.

Arias-Alzate, A., Downer, C. C., Delgado, C. A., Sánchez-Londoño, J. D. (2010). Un registro de Tapir de montaña (*Tapirus pinchaque*) en el norte de la Cordillera Occidental de Colombia. *Mast. Neotrop.* 17: 111-116.

Arispe, R., Rumiz, D. I., Venegas C. (2007). Censo de jaguares (*Panthera onca*) y otros mamíferos con trampas cámara en la concesión temporal forestal El Encanto. Technical Inform 173. Wildlife Conservation Society, Santa Cruz de la Sierra, Bolivia. Pp. 1-37.

Ascunce, M.S., Hasson, E., Mudry, M.D. (2003). COII: a useful tool for inferring phylogenetic relationships among New World monkeys (Primates, Platyrrhini). *Zool. Scripta* 32: 397-406.

Ayala, G. (2003). *Monitoreo de Tapirus terrestris en el Izozog (Cerrado Cortado), mediante el uso de telemetría como base para un plan de conservación*. Master Thesis. Universidad Mayor de San Andrés, La Paz, Bolivia. Pp. 1-96.

Bandelt, H-J., Forster, P., Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37-48.

Barrientos, J., Cuéllar, E. (2003). Radiotelemetria de antas (*Tapirus terrestris*) en el Chaco seco, Izozog, Santa Cruz, Bolivia. In Manejo de Fauna Silvestre en Amazonía y Latinoamérica. Polanco-Ochoa, R (Edit.). CITES, Fundación Natura, Bogotá DC., Colombia. Pp. 1-446.

Bertorelle, G., Barbujani, G. (1995). Analysis of DNA Diversity by Spatial Autocorrelation. *Genetics* 140: 811-819.

Bodmer, R. E., Brooks, D. M. (1997). Status and action plan of the lowland tapir (*Tapirus terrestris*). In Brooks, D. M., Bodmer, R. E., Matola, S., (Eds). *Tapir-status survey and conservation action plan*. IUCN/SSC Tapir Specialist Group, IUCN Gland & Cambridge.Pp. 46-56.

Bodmer, R. E., Aquino, R., Puertas, P. (1997a). Alternativas de Manejo para la Reserva Nacional Pacaya-Samiria: Un análisis sobre el uso sostenible de la caza. In *Manejo de la Fauna Silvestre en la Amazonia*. Fang, T. G., Bodmer, R. E., Aquino, R. & Valqui, M. H. (Eds.). Instituto de Ecologia, La Paz, Bolivia. Pp.65-74.

Bodmer, R. E., Puertas, P. E., Reyes, C., García, J. E., Diaz, D. (1997b). Animales de caza y palmeras: Integrando la socio-economía de extracción de frutos de palmera y carne de monte con el uso sostenible. In *Manejo de la Fauna Silvestre en la Amazonia*. Fang, T. G., Bodmer, R. E., Aquino, R. & Valqui, M. H. (Eds.). Instituto de Ecologia, La Paz, Bolivia. Pp.75-86.

Bodmer, R. E., Aquino, R., Navarro, J. (2000). Sustentabilidad de la caza de mamíferos en la cuenca del río Samiria, Amazonía Peruana. In Cabrera, E., Mercolli, C. & Resquin, R. (Eds.). *Manejo de Fauna Silvestre en Amazonía y Latinoamérica*. CITES, Paraguay, Fundación Moises Bertoni & University of Florida, Asunción, Paraguay. Pp. 447-449.

Bradley, R.D., Baker, R.J. (2001). A test of the genetic species concept: cytochrome-b sequences and mammals. *J. Mammal.* 82: 960–973.

Brehm, G. L., Pitkin, M., Hilt, N., Fiedler, K. (2005). Montane Andean rain forests are a global diversity hotspot of geometrid moths. *J. Biogeog*. 32: 1621-1627.

Brown Jr., K. S., Sheppard, P. M., Turner, J. R. G. (1974). Quaternary refugia in tropical America: evidence from race formation in *Heliconius* butterflies. *Proc. R. Soc. Lond. B.* 187: 369-378.

Cabrera A. (1961). Catálogo de los mamíferos de América del Sur. *Revista del Museo Argentino de Ciencias Naturales. Ciencias Zoológicas* 4: 309 – 732.

Carroll, R. L. (1988). Ungulates, Edentates and Whales. In Carroll, R. L. (Ed.). *Vertebrate Paleontology and Evolution*. Freeman, New York. Pp. 502–568.

Cavelier, J., Etter, A. (1995). Deforestation of montane forest in Colombia as result of illegal plantations of opium (*Papaver somniferum*). In Churchill, S. P., Balsley, H., Forero, E., Luteyn, J. L. (Eds.). *Biodiversity and Conservation of Neotropical montane forest*. New York Botanical Garden, New York. Pp. 125-137.

Cavelier, J., Lizcano, D., Yerena, E., Downer, 967 C. (2010). The mountain tapir (*Tapirus pinchaque*) and Andean bear (*Tremarctos ornatus*): Two charismatic, large mammals in South American tropical montane cloud forests. In Bouijnacel L. A., Scabena, F. N., Hamilton, L. S (Eds.). *Tropical Montane Cloud Forests*: *Science for Conservation and Management*. Cambridge University Press, Cambridge, USA. Pp. 172-181.

Chalukian, S. C., de Bustos, M. S., Lizárraga, L., Varela, D., Paviolo, A., Juliá, J. P., Quse, V. (2013). Plan de Acción para la conservación del Tapir (*Tapirus terrestris*) en Argentina: Estado de Conservación y Perspectivas. Libro de Resúmenes del I Congreso Latinoamericano de Tapires y II Congreso Ecuatoriano de Mastozoología, Puyo, Ecuador, May 8-11, 2013. Pp. 95-96.

Chamorro-Rengifo, J., Cubillos-Rodriguez, P. A. (2007). Fichas de Fauna y Flora proyecto "Biodiversidad y desarrollo en ecoregiones estratégicas de Colombia-Orinoquía". Sistema de Información sobre Biodiversidad de Colombia (SIB), Bogotá, Colombia. http://www.siac.net.co/sib /catalogoespecies.

Clapperton, C. (1993). *Quaternary geology and geomorphology of South America*. Elsevier, Amsterdam, The Nederlands. Pp. 1-489.

Colbert, M. (2005). The facial skeleton of the Early Oligocene *Colodon* (Perissodactyla, Tapiroidea). *Palaeontologia Electronica* 8: 1-27.

Collins AC, Dubach JM. (2000). Biogeographic and Ecological forces responsible for speciation 916 in *Ateles*. *Int. J. Primat.* 21: 421-444.

Constantino E., Lizcano D., Montenegro O., Solano C. (2006). Danta Común. In: Libro Rojo de los Mamíferos de Colombia. Conservación Internacional Colombia, Ministerio de Ambiente, Vivienda y Desarrollo Territorial. Bogotá, Colombia. Pp. 1-433.

Cossíos, E.D., Lucherini, M., Ruiz-García, M., Angers B. (2009). Influence of ancient glacial periods on the Andean fauna: the case of the Pampas cat (*Leopardus colocolo*). *BMC Evolutionary Biology* 9: 68-79.

Cossíos, E.D, Walker S, Lucherini M, Ruiz-García M, Angers B. (2012). Between high-altitude islands and high-altitude corridors. The population structure of the Andean cat (*Leopardus jacobita*). *Endangered Species Research* 16: 283-294.

De Wilde, A.H. (1994). Caramanta, un proyecto para la creación de un nuevo parque nacional natural. Pp.33, In Cavelier, J., Uribe, A (eds.). Resúmenes del Simposio Nacional―Diversidad biológica, conservación y manejo de los ecosistemas de montaña en Colombia. Universidad de los Andes, Bogota, Colombia.

Deng, T. (2006). Paleoecological comparison between late Miocene localities of China and Greece based on *Hipparion* faunas. *Geodiversitas* 28: 499-516.

Dobzhansky, T. (1937). *Genetics and the Origin of Species*. Columbia University Press, New York, USA. Pp. 1-364.

Dobzhansky, T. (1971). Evolutionary oscillations in Drosophila pseudoobscura. In: Creed, R. (Eds.). *Ecological genetics and evolution*. Blackwell Scientific, Oxford, UK. Pp. 109–133.

Downer, C.C. (1996). The mountain tapir, endangered 'flagship' species of the high Andes. *Oryx* 30: 45-58.

Downer, C.C. (1997). Status and action plan of the mountain Tapir (*Tapirus pinchaque*). Tapirs, status survey and conservation action plan (eds D.M. Brooks, R.E. Bodmer and S. Matola), pp. 10-22. IUCN/SSC Tapir specialist group, IUCN, Gland, Switzerland and Cambridge, UK.

Downer, C. C. (2003). Ámbito hogareño y utilización de hábitat del Tapir Andino e ingreso de ganado en el Parque Nacional Sangay, Ecuador. *Lyonia* 4: 31-34.

East, R. (1981). Species–area curves and populations of large mammals in African savanna reserves. *Biological Conservation* 21: 111–126.

Eldredge, N., Gould, S. J. (1972). Punctuated equilibria: An alternative to phyletic gradualism. Schopf, T. J. M. (Ed.) in *Models in Paleobiology*. Ed. Freeman, San Francisco. Pp. 82-115.

Excoffier, L., Smouse, P.E., Quattro, J.M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

Excoffier, L., Laval, G. & Schneider, S. (2005). Arlequin (version 3.0): An integrated software Packaged for population genetics data analysis. *Evolutionary Bioinformatics* 1: 47 –50.

Ferrero, B. S., Noriega, J. I. (2007). A new upper Pleistocene tapir from Argentina: Remarks on the phylogenetics and diversification of neotropical Tapiridae. *Journal of Vertebrate Paleontology* 27: 504-511.

Fjeldsa, J. (1994). Geographical patterns for relict and young species of birds in Africa and South America and implications for conservation priorities. *Biodiversity Conserv.* 3: 207-226.

Forasiepi, A., Martinelli, A., Blanco J. (2007). Bestiario Fósil. Mamíferos del Pleistoceno de la Argentina. Editorial Albatros SACI, Buenos Aires, Argentina. Pp. 1-190.

Froehlich, D. J. (1999). Phylogenetic systematics of basal perissodactyls. *Journal of Vertebrate Paleontology* 19: 140-159.

Fu, Y-X. (1997). Statistical tests of neutrality against population growth, hitchhiking and background selection. *Genetics* 147: 915-925.

Fu, Y., Li, W. (1993). Statistical Tests of Neutrality of Mutations. *Genetics* 133: 693-709.

Gabriel, K.R., Sokal, R.R. (1969). A new statistical approach to geographic variation analysis. *Systematic Zoology* 18: 259-278.

Goldman, N., Anderson, P., Rodrigo, A.G. (2000)**.** Likelihood-based tests of topologies in phylogenetics. *Systematic Biology* 49: 652-670.

Gould, S. J., and Eldredge, N. (1993). Punctuated equilibrium comes of age. *Nature* 366: 223-227.

Groves C. P., Fernando, P., Robovsky, J. (2010). The sixth Rhino: A taxonomic reassessment of the critically endangered northern White rhinoceros. *PLoS ONE* 5: e9703.

Haffer, J. (1969). Speciation in Amazonian forest birds. *Science* 165: 131-137.

Haffer, J. (1970). Geologic-climatic history and zoogeographic significance of the Uraba region in northwestern Colombia. *Caldasia* 10: 603-636.

Haffer, J. (1974). Avian speciation in tropical South America. *Publ. Nuttall. Ornith. Club*. 14: 1-390.

Haffer, J. (1982). General aspects of the refuge theory. In Prance, G. T., (Ed.). *Biological diversification in the tropics.* New York: Columbia University. Pp. 6-24.

Haffer, J. (1987). Quaternary history of tropical America. In Whitmore, T.C., and Prance, G.T. (Eds.). *Biogeography and Quaternary history in tropical America*. Oxford: Clarendon and Oxford University Press. Pp. 1-18.

Haffer, J. (1997). Alternative models of vertebrate speciation in Amazonia: an overview. *Biodiversity and Evolution* 6: 451-476.

Haffer, J. (2008). Hypotheses to explain the origin of species in Amazonia. *Braz. J. Biol.* 68: 917-947.

Hall, T. (2004). Bioedit Sequence Alignment Editor. Version 7.0.0.

Harpending, H.C. (1994). Signature and ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biol*. 66: 591-600.

Harpending, H.C., Sherry, S.T., Rogers, A.R., Stoneking, M. (1993). Genetic structure of ancient human populations. *Current Anthrop*. 34: 483-496.

Hebert, P.D.N., Ratnasingham, S., de Waard, J.R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond. B* 270 (Suppl.): S96-9.

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T., Francis, C.M. (2004). Identification of birds through DNA barcodes. *PLoS Biol.* 2: 1657-63.

Henderson, A., Churchill, P., Luteyn, J. (1991). Neotropical plant diversity. *Nature* 229: 44–45.

Hershkovitz, P. (1954). Mammals of Northern Colombia. Preliminary Report no. 7. Tapirs (genus *Tapirus*), with a Systematic Review of American Species. *Proceedings of the United States National Museum* 103: 465-496.

Holanda, E. C., Rincón, A. D. (2012). Tapirs from the Pleistocene of Venezuela. *Acta Palaeontologica Polonica* 57: 463-472.

Holanda, E. C., Ferrero, B. C. (2013). Reappraisal of the genus *Tapirus* (Perissodactyla, Tapiridae): Systematics and phylogenetic affinities of the South American tapirs. *Journal of Mammalian Evolution* 20: 33-44.

Holbrook, L. T. (1999). The phylogeny and classification of tapiromorph perissodactyls (Mammalia). *Cladistics* 15: 331-350.

Hudson, R.R. (2000). A new statistic for detecting genetic differentiation. Genetics 155: 2011-2014.

Hudson, R.R., Boss, D.D., Kaplan, N.L. (1992a). A statistical test for detecting population subdivision. *Molecular Biology and Evolution* 9: 138-151.

Hudson, R.R., Slatkin, M., Maddison, W.P. (1992b). Estimations of levels of gene flow from DNA sequence data. *Genetics* 132: 583-589.

Huelsenbeck, J.P., Bull, J.J. (1996). A likelihood ratio test to detect conflicting phylogenetic signal. *Systematic Biology* 45: 92-98.

Hulbert, R. C. (1995). The giant tapir, *Tapirus haysii*, from Leisey Shell Pit 1A and other Florida Irvingtonian localities. *Bull. Fla. Mus. Nat. Hist.* 37: 515-551.

Hulbert, R. C. (2010). A new early Pleitocene tapir (Mammalia: Perissodactyla) from Florida, with a review of Blancan tapirs from the state. *Bulletin of the Florida Museum of Natural History* 49: 67–126.

Hulbert, R. C., Wallace, S. C. (2005). Phylogenetic analysis of late Cenozoic *Tapirus* (Mammalia, Perissodactyla). *Journal of Vertebrate Paleontology* 25 (supplement to 3): 72A

Hulbert, R. C., Wallace, S. C., Klippel, W. E., Parmalee, P. W. (2009). Cranial morphology and systematics of an extraordinary sample of the late Neogene Dwarf Tapir, *Tapirus polkensis* (Olsen). *Journal of Paleontology* 83: 238-262.

Isaaks EH, Srivastava RM. (1989). *An introduction to applied geostatistics*. Oxford University Press, New York. Pp 1-561.

Kartavtsev, Y. (2011). Divergence at Cyt-b and Co-1 mtDNA genes on different taxonomic levels and genetics of speciation in animals. Mitochondrial DNA 22: 55-65.

Klammer, G. (1984). The relief of the extra-Andean Amazon basin. In Sioli, H. (Ed.). *The Amazon. Limnology and landscape ecology of a mighty tropical river and its basin.* Dordrecht: Junk Publishers. Pp. 47-83.

Kelly J. (1997). A test of Neutrality Based on Interlocus Associations. *Genetics* 146: 1197-1206.

Kessler, M. (2002). The elevational gradient of Andean plant endemism: varying influences of taxon-specific traits and topography at different taxonomic levels. *J. Biogeog.*29: 1159-1165.

Kimura M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16: 111-120.

Krömer, T., Kessler, M., Gradstein, S. R., Acebey, A. (2005). Diversity patterns of vascular epiphytes along an elevational gradient in the Andes. *J. Biogeogr* 29: 1159-1165.

León-Canales, E. (2007). *Origenes humanos en los Andes peruanos*. Universidad de San Martín de Porres. Lima, Perú. Pp. 1-328.

Librado, P., Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451-1452 | doi: 10.1093/bioinformatics/btp187.

Lizcano, D.J., Cavelier, J. (2000). Densidad poblacional y disponibilidad de habitat de la danta de montaña (*Tapirus pinchaque*) en los andes centrales de Colombia. *Biotropica* 31: 165-173.

Lizcano, D. Pizarro, J. V., Cavelier, J., Carmona, J. 2002. Geographic distribution and population size of the mountain tapir (*Tapirus pinchaque*) in Colombia. *J. Biogeog*. 28: 1-9.

MacFadden, B. J. (1992). *Fossil horses: Systematics, paleobiology, and evolution of the family Equidae*. Cambridge University Press, New York.

MacNeish, R. S. (1979). The early man remains from Pikimachay cave, Ayacucho basin, highlands Peru. In Humprey, R. L. & Standford, D., (Eds.). *Pre-Llano cultures of the Americas: Paradoxes and possibilities*. Anthropological Society of Washington, Pp. 1-47.

Mantel, N.A. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209-220.

Matula, D.W., Sokal, R.R. (1980). Properties of Gabriel graphs relevant to geographic variation research and the clustering of points in the plane. *Geographic Analysis* 12: 205-222.

Marroig, G., Cerqueira, R. (1997). Plio-Pleistocene South American history and the Amazon Lagoon hypothesis: a piece in the puzzle of Amazonian diversification. *J. Comp. Biol.* 2: 103-119.

McKenna, M. C., Bell, S. K. (1997). *Classification of Mammals-Above the species level*. Columbia University Press, New York. Pp. 631.

Meffe GK, Carroll CR. (1997). *Principles of Conservation Biology*. Second Edition. Sinauer Associates, INC. Publishers. Sunderland, Massachusetts.

Merelender, A. M., Woodruff, D. S., Ryder, O. A., Kock, R., Vahala, J. (1989). Allozyme variation and differentiation in African and Indian rhinoceroses. *J. Hered.* 80: 377–382.

Metais, G., Soe, A. N., Ducrocq, S. (2006). A new basal tapiromorph (Perissodactyla, Mammalia) from the middle Eocene of Myanmar. *Geobis* 39: 513-519.

Metivier, S. P. (1998). *A reconstruction of glacial extent, temperature and precipitation in South America at the time of the Last Glacial Maximum*. PhD Thesis, Syracuse University, Syracuse, USA. Pp. 1-116.

Montenegro, O. (2002). Evaluación del estado actual de la danta o tapir de páramo (*Tapirus pinchaque*) en la región Andina Oriental, con base en una recopilación y verificación de registros de campo y una aproximación preliminar al estado de su hábitat en la región. Informe final. CORPOCHIVOR, CAR, CORPOGUAVIO, CORPOBOYACA & Ministerio del Medio Ambiente. Garagoa. Colombia.

Morales, J. C., Melnick, D. J. (1994). Molecular systematics of the living rhinoceros. *Mol. Phylogenet. Evol.* 3: 128–134.

Moritz, C., Faith, D.P. (1998). Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology* 7:419-429.

Morral, N., Bertrantpetit, J., Estivill, X., et al., (1994). The origin of the major cystic fibrosis mutation (delta F508) in European populations. *Nature Genetics* 7: 169-175.

Nabholz, B., Glemin, S., Galtier, N. (2008). Strong variations of mitochondrial mutation rate 1224 across mammals—the longevity hypothesis. *Mol. Biol. Evol.* 25: 120–130.

Nabholz, B., Glemin, S., Galtier, N. (2009). The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. *BMC Evol. Biol*. 9: 54-67.

Nei, M., Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press, New York. Pp.1-333.

Nores, M. (1999). An alternative hypothesis for the origin of Amazonian bird diversity. *J. Biogeogr*. 26: 475-485.

Nores, M. (2004). The implications of Tertiary and Quaternary sea level rise events for avian distribution patterns in the lowlands of northern South America. *Global Ecology and Biogeography* 13: 149-162.

Noriega, J., Carlini, A. A., Brandoni, O., Ferrero, B. S., Vassalo, C., Cettour de Soto, S. (2004). Mamíferos del Cuaternario de la cuenca del río Uruguay, Departamento de Concordia, Entre Ríos, Argentina. Reu. An. de Comunic. APA, Resúmenes, Diamante, APA. Pp. 21-22.

Norman, J., Ashley, M. (2000). Phylogenentics of Peryssodactyla and Tests of the Molecular Clock. *Journal of Molecular Evolution* 50: 11-21.

Oden, N. (1984). Assessing the significance of a spatial correlogram. Geographical Analysis 16: 1-16.

Orlando, L., Ginolhac, A., Zhang, G., et al., (2013). Recalibrating *Equus* evolution using the genome sequence of an early Middle Pleistocene horse. *Nature* 499: 74-81.

Padilla, M., Dowler, R. C. (1994). *Tapirus terrestris*. *Mammalian species* 481: 1-8.

Perini, A.F., Oliveira, J.A., Salles, L.O., Moraes Neto, C.R., Guedes, P.G., Oliveira, L.F.B., Weksler, M. (2011). New fossil records of *Tapirus* (Mammalia, Perissodactyla) from Brazil, with a critical analysis of intra-generic diversity assessments based on lower molar size variability. *Geobios* 44: 609-619.

Pimm SL. (1991). The balance of nature? Ecological issues in the conservation of species and communities. University of Chicago Press, Chicago.

Posada D, Crandall KA. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

Prance, G. T. (1982). *Biological diversification in the tropics*. New York: Columbia University.

Prance, G. T. (1996). Islands in Amazonia. *Phil. Trans. R. Soc. Lond. B* 351: 823-833.

Prance, G.T., Lovejoy, T.E. (1985). *Amazonia. Key environments.* Oxford: Pergamon Press.

Rabinowitz, A., Zeller, K. A. 2010. A range-wide model of landscape connectivity and conservation for the jaguar, *Panthera onca*. *Biological Conservation* 143: 939-945.

Radinsky, L. B. (1969). The early evolution of the Perissodactyla. Evolution 23: 308-328.

Rambaut, A., Grassly, N. C. (1997). Seq-gen: an application for the monte carlo simulation of DNA sequence evolution along phylogenetic trees. *Computer Applications in the Biosciences* 13: 235-238.

Ramos-Onsins, S.E., Rozas, J. (2002). Statistical Properties of New Neutrality Tests Against Population Growth. *Mol. Biol. Evol*. 19: 2092–2100.

Ripley, B.D. (1981). *Spatial statistics*. John Wiley and Sons, New York.

Rodbell, D. T. (1991). *Late Quaternary glaciation and climatic change in the northern Peruvian Andes*. PhD Thesis. University of Colorado, Boulder, Colorado, USA. Pp. 1-416.

Rodbell, D. T., Seltzer, G. O. (2000). Rapid ice margin fluctuations during the Younger Dryas in the tropical Andes. *Quaternary Research* 54: 328-338.

Rohlf, F.J. (2000). *NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System, Version 2.1*. Exeter Publications, New York, USA.

Rogers, A.R., Harpending, H.C. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evol*. 9: 552-569.

Rogers, A.R., Fraley, A.E., Bamshad, M.J., Watkins, W.S., Jorde, L.B. (1996). Mitochondrial mismatch analysis is insensitive to the mutational process. *Mol. Biol. Evol*. 13: 895-902.

Romero, V. P., Ferrer A., Lew D. (2013). La danta de tierras bajas como principal fuente proteica en una comunidad agrícola al Sur del río Orinoco, Venezuela: Implicaciones para su conservación. Libro de Resúmenes del I Congreso Latinoamericano de Tapires y II Congreso Ecuatoriano de Mastozoología, Puyo, Ecuador, May 8-11, 2013. Pp. 103.

Rothlisberger, F. (1987). *10,000 jahre gletschergeschichte der erde*. Verlag Sauerlander, Aarau, Switzerland. Pp. 1-225.

Rozas, J., Gullaud, M., Blandin. G., Aguadé, M. (2001). DNA Variation at the rp49 Gene Region of *Drosophila simulans*: Evolutionary Inferences from an Unusual Haplotype Structure. *Genetics* 158: 1147-1155.

Ruiz-García, M. (2003). Molecular population genetic analysis of the spectacled bear (*Tremarctos ornatus*) in the Northern Andean Area. *Hereditas* 138: 81-93.

Ruiz-García, M. (2013). The genetic demography history and phylogeography of the Andean bear (*Tremarctos ornatus*) by means of microsatellites and mtDNA markers. In *Molecular Population Genetics, Evolutionary Biology and Conservation of Neotropical*

Carnivores. Ruiz-García, M., Shostell, J. (Eds.). Nova Science Publishers. New York. Pp. 129-158.

Ruiz-García, M., Orozco-terWengel, P., Payán, E., Castellanos, A. (2003). Genética de Poblaciones molecular aplicada al estudio de dos grandes carnívoros (*Tremarctos ornatus* – Oso andino, *Panthera onca*- jaguar): lecciones de conservación. *Bol. Real Soc. Esp. Hist. Nat.* 98: (1-4): 135-158.

Ruiz-García, M., Orozco-terWengel, P., Castellanos, A., Arias, L. (2005). Microsatellite analysis of the spectacled bear (*Tremarctos ornatus*) across its range distribution. *Genes and Genetics Systems* 80: 57-69.

Ruiz-García, M., Vásquez, C., Pinedo-Castro, M., Sandoval, S., Kaston, F., Thoisy, B., Shostell, J. M. (2012). Phylogeography of the mountain tapir (*Tapirus pinchaque*) and the Central American tapir (*Tapirus bairdii*) and the molecular origins of the three South-American tapirs. In Anamthawat-Jónsson, K. (Ed.). *Current Topics in Phylogenetics and Phylogeography of Terrestrial and Aquatic Systems*. InTech, Rijeka, Croatia. Pp. 83-116.

Ruiz-García, M., Cossíos, D., Lucherini, M., Yañez, F., Pinedo-Castro, M., Angers, B. (2013). Population genetics and spatial structure in two Andean cats (the Pampas cat, *Leopardus pajeros* and the Andean mountain cat, *L. jacobita*) by means of nuclear and mitochondrial markers and some notes on skull biometrics. Ruiz-García, M., and Shostell, J. M., (Eds.) in *Molecular Population Genetics, Evolutionary Biology and Biological Conservation of Neotropical Carnivores*. Nova Science Publishers, Inc.. New York (USA). Pp. 187-246.

Ruiz-García, M., Vásquez, C., Sandoval, S., Kaston, F., Luengas-Villamil, K., Shostell, J. M. (2015a). Phylogeography and spatial structure of the lowland tapir (*Tapirus terrestris*, Perissodactyla: Tapiridae) in South America. *Mitochondrial DNA* 26: 1-9, on line DOI: 10.3109/19401736.2015.1022766

Ruiz-García, M., Castellanos, A., Bernal, L. A., Pinedo-Castro, M., Kaston, F., Shostell, J.M. (2015b). Mitogenomics of the elusive mountain tapir (*Tapirus pinchaque*, Tapiridae, Perissodactyla, Mammalia) in Colombia and Ecuador: Phylogeography and insights into the origin and systematics of the South American tapirs. *J. Hered.* (in press).

Ruiz-García, M., Pinedo-Castro, M., Shostell, J. M. (2015c). Is "*Tapirus kabomani*" a new Amazonian tapir species? The fulminant rise and fall of a "typological species" by means of mitogenomics and craniometrics. *J. Mammal*. (submitted).

Saillard, J., Forster, P., Lynnerup, N., Bandelt, H-J., Norby, S. (2000). mtDNA variation among Greenland Eskimos: the edge of the Beringian expansion. *American Journal of Hum. Genet.* 67: 718-726.

Salazar-Bravo, J., Tarifa T., Aguirre, L. F., Yensen, E., Yates, T. L. (2003). Revised checklist of Bolivian Mammals. *Occasional Papers, Museum of Texas Tech University*, 220: 1-27.

Sambrock, J., Fritsch, E., Maniatis, T. (1989). *Molecular Cloning: A Laboratory manual*. 2nd edition. V1. Cold Spring Harbor Laboratory Press. New York.

Sarriá, S. (1993). Parque Nacional Natural Farallones de Cali. Monografía. Corporación Autónoma Regional del Valle del Cauca-CVC. Fundación Protectora de las Cuencas-PROCUENCAS. Cali, Colombia. Pp. 1-312.

Savage, R. J. G., Long, M. R. (1986). *Evolución de los Mamíferos: Una guía ilustrada*. Ediciones Akal, Torrejón de Ardoz, Madrid. Pp. 1-258.

Schaller, G. B. (1983). Mammals and their biomass on a Brazilian ranch. *Arquivos de Zoologia, Sao Paulo* 31: 1-36.

Seltzer, G. O. (1987). *Glacial history and climatic change in the central Peruvian Andes*. Msc Thesis, University of Minnesota, Minneapolis, Minessota, USA. Pp. 1-276.

Seltzer, G. O. (1990). Recent glacial history and paleoclimate of the Peruvian-Bolivian Andes. *Quaternary Science Reviews* 9: 137-152.

Shimodaira, H., Hasegawa, H. (1999). Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* 16: 1114-1116.

Simonsen, K., Churchill, G., Aquadro, C. (1995). Properties of Statistical Tests of Neutrality for DNA Polymorphism Data. *Genetics* 141: 413-429.

Smouse, P.E., Long, J.C., Sokal, R.R. (1986). Multiple regression and correlation extension of the Mantel test of matrix corresponde. *Systematic Zoology* 35: 627-632.

Sokal, R.R., Oden, N.L. (1978). Spatial autocorrelation in Biology. 1. Methodology. *Biological Journal of Linnean Society* 10: 199-228.

Sombroek, W. G. (1966). *Amazon soils.* Wageningen: Centrum voor Landbouwpublikaties.

Sphuler, J. N. (1972). Genetic, linguistic, and geographical distances in native North America. In *The assessment of population affinities in man.* Wiener, J. S., Huizinga, J (Eds.). Oxford University Press, Oxford.

Swofford, D. L, Olsen, G. L, Wadell, P. J, Hillis, D. M. (1996). Phylogenetic inference. In Hillis, D.M., (Ed.) *Molecular Systematics*. Sunderland, Massachusetts: Sinauer Associates. Pp. 407-514.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585-595.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28: 2731-2739.

Terborgh, J. (1992). *Diversity and the tropical rain forest.* New York: Freeman.

Thoisy, B., Goncalves da Silva, A., Ruiz-García, M., Tapia, A., Ramirez, O., Arana, M., Quse, V., Paz-y-Miño, C., Tobler, M., Pedraza, C., Lavergne, A. 2010. Population history, phylogeography, and conservation genetics of the last Neotropical mega-herbivore, the Lowland tapir (*Tapirus terrestris*). *BMC Evol. Biol.* 10: 278-295.

Thompson, L. G., Mosley, E., Davies, M. E., Lin, P. N., Henderson, K. A., Coledal, J., Bolzan, J. F., Liu, K. B. (1995). Huascarán, Perú. *Science* 269: 46-50.

Tirira, D. (2011). *Libro Rojo de los Mamíferos del Ecuador*. Publicación especial de los Mamíferos del Ecuador No 8. Pontificia Universidad Católica del Ecuador and Ministerio de Medio Ambiente, Quito, Ecuador.

Tirira, D., Castellanos, A. (2001). Tapir de montaña (*Tapirus pinchaque*). In Tirira D (Ed.). *Libro Rojo de los Mamiferos del Ecuador*. SIMBIOE/EcoCiencia/Ministerio del Ambiente/UICN. Serie Libros Rojos del Ecuador. Tomo 1. Publicación Especial sobre los Mamíferos del Ecuador 4, Quito. Pp. 98-100.

Tonni, E. P. (1992). *Tapirus* Brisson, 1762 (Mammalia, Perissodactyla) en el Lujanense (Pleistoceno Superior-Holoceno Inferior) de la Provincia de Entre Ríos, Republica Argentina. *Ameghiniana* 29: 3-8.

Tougard, C., Delefosse, T., Hänni, F., Montgelard, C. (2001). Phylogenetics relationships of the five extant rhinoceros species (Rhinocerotidae, Perissodactyla) based on mitochondrial Cytochrome b and 12S rRNA genes. *Molecular Phylogenetics and Evolution* 19: 34-44.

Upton G, Fingleton B. (1985). *Spatial data analysis by example*. Vol 1: Point pattern and quantitative data. John Wiley and Sons, Chichester.

Van der Hammen, T. (1992). *Historia, ecología y vegetación.* Editorial Corporación Colombiana para la Amazonía, Araracuara, Bogotá DC., Colombia. Pp. 1-411.

Van der Hammen, T. (2001). Paleoecology of Amazonia. In: Guimaraes Vieira, I. C., Silva, J. M. C., Oren, D. C. & D'Incao, M. A. D. (Eds). *Diversidade biológica e cultural da Amazonia*. Museu Paraense Emilio Goeldi, Belem, Brazil. Pp. 19-44.

Van der Hammen, T., Cleff, A. M. (1992). Holocene changes of rainfall and river discharge in northern South America and the El Niño phenomenon. *Erdkunde* 46: 252-256.

Vanzolini, P. E. (1970). *Zoología sistemática, geografía e a origem das espécies.* São Paulo: Instituto Geográfico de São Paulo. Pp. 1-56.

Vanzolini, P. E. (1973). Paleoclimates, relief, and species multiplication in equatorial forests. In Meggers, B. J., Ayensu, E.S., Duckworth, W.D. (Eds.). *Tropical forest ecosystems in Africa and South America: A comparative review.* Washington: Smithsonian Institution. Pp. 255-258.

Vanzolini, P. E. (1992). Paleoclimas e especiacao em animais da América do Sul tropical. São Paulo. *Estud. Avancados* 6: 41-65.

Vanzolini, P.E., Williams, E. E. (1970). South American anoles: Geographic differentiation and evolution of the *Anolis chrysolepis* species group (Sauria, Iguanidae). *Arq. Zoolog* 19: 1-298.

Verweij, P. A. (1995). Spatial and temporal modelling of vegetation patterns. International Institute for Aerospace Survey and Earth Sciences ITC. Enschede, The Netherlands.

Voss, R.S., Helgen, K. M., Jansa, S. A. (2014). Extraordinary claims require extraordinary evidence: A comment on Cozzuol et al. (2013). *J Mammal* 95:893–8.

Wallace, R. B., Gómez, H., Porcel, Z. R., Rumiz, D. I. (2010). *Distribución, Ecología y Conservación de los Mamíferos medianos y grandes de Bolivia*. Editorial Centro de Ecología Difusión Fundación Simón I. Patiño, Santa Cruz de la Sierra, Bolivia. Pp. 1-906.

Walsh, P.S., Metzger, D. A., Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* 10: 506-513.

White, M.J.D. (1968). Models of speciation. New concepts suggest that the classical sympatric and allopatric models are not the only alternatives. *Science* 159: 1065-1070.

White, M.J.D. (1978). *Modes of speciation*. W. H. Freeman, San Francisco.

Whithmore, R. T., Prance, G. T. (1987). Biogeography and quaternary history in tropical America. Oxford: Clarendon Press. (Monograpy on Biogeography, 3).

Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics* 15: 323–354.

Xu, X. (1996). Studies of mammalian mitochondrial genomes with special emphasis on the Perissodactyla. Ph.D. dissertation, University of Lund, Sweden.

Yamini, B., Schillhorn, T. W. (1988). Schistosomiasis and nutritional myopathy in a Brazilian tapir (*Tapirus terrestris*). *Journal of Wildlife Diseases* 24: 703-707.

Zeller, K. A., Rabinowitz, A., Salom-Pérez, R., Quigley, H. (2013). The jaguar corridor initiative: A range-wide conservation strategy. In Ruiz-García, M., Shostell, J. M. (Eds.). *Molecular Population Genetics, Evolutionary Biology and Biological Conservation of Neotropical Carnivores.* Nova Science Publishers, Inc., New York, USA. Pp. 629-657.